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MELANOMA STUDIES *

I. THE DOPA REACTION IN GENERAL PATHOLOGY

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Introduced in 1917 by the distinguished dermatologist of Zurich, Bruno Bloch, [1-4] and defended by him vigorously ever since, [5-15] the dopa reaction has been used chiefly by the dermatopathologist (Becker,[16, 17] Peck, [18-20] and Kissmeyer [21, 22]). It deserves to be more widely known and practiced both by the histologist and by the general pathologist. Following the simple method devised by Blackberg, which is described in detail in the second paper of this series, the dopa reaction ** can be carried out easily and accurately in any laboratory.

The dopa reaction is specific for two kinds of cells, for melanoblasts (a term which includes all melanin-producing cells as distinguished from mere phagocytes), and for myelogenous leucocytes (cells which have no known connection with melanin production). Both of these cells contain a ferment, an oxidase, which converts dopa to melanin. The newly formed melanin colors the cell black. This blackening of the reacting cell is the dopa reaction. The ferment of the melanoblast is dopa-oxidase — specific for dopa — it oxidizes nothing else. The ferment of the leucocyte is a polyphenol-oxidase, oxidizing many phenols to colored products.

Figure 1 shows the dopa reaction of a Hodgkin's lymph node. The black spots are the eosinophil and neutrophil leucocytes. Lymphocytes and collagen are colorless.

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** The word "dopa" is Bloch's abbreviation for 3, 4-dioxyphenylalanin.

Figure 2 shows the dopa reaction of normal Caucasian skin. In the colorless derma are a few black leucocytes. In most sections of normal skin there are a few scattered leucocytes; they have no significance. The chief point of interest is the blackening of certain cells in the basal layer of the epidermis and in the matrix of the hair — that is to say — the sites of melanin production. These are the melanoblasts revealed by the dopa reaction.

The melanoblasts of the epidermis assume many shapes — round, cuboidal, columnar and branched. Traditionally the branched cells of the epidermis are called dendritic. Often they contain melanin, often not. Their common property is the ability to oxidize dopa to melanin, blackening in dopa solutions. Bloch holds that in this dopa-oxidase which converts dopa to melanin he has discovered the agent which manufactures natural melanin in mammalian skin. The dopa reaction thus becomes an indicator of the presence of the natural oxidase. Leucocytes being excepted, it follows that every dopa-positive cell is an active melanoblast.

After many experiments on all sorts of tissue, normal and pathological, pigmented and non-pigmented, I accept Bloch's view as the most satisfactory working hypothesis yet offered to explain the production of melanin in human skin. The illustrations will show some of the evidence which led to this conclusion.

Our material consisted of freshly excised surgical specimens from many parts of the human body. Our first and fundamental conclusion was that, leucocytes being excepted, dopa-positive cells are found only in those tissues where melanin is being produced, or where it can be produced under appropriate stimulation. These tissues normally are the skin and the mucous membranes of ectodermal origin; pathologically they are pigmented moles and malignant melanomas.*

Melanin in Excess

Having agreed with Bloch that dopa-positive cells are found only in melanin-producing tissue, in our next finding we were obliged to

* The pigment of the eye constitutes a special field which has been little cultivated. Since the dopa reaction requires fresh tissue, or tissue that has been fixed in 5 per cent formalin for five hours at the longest; and since it is customary to fix specimens of human eye much longer than that, there are practical difficulties in attempting dopa reactions with this material. A thorough study of the dopa reaction of the embryonic eye was made by Miescher.[33]

agree with him again that wherever melanin is being produced in excess, there the dopa-positive cells are increased in number and often in complexity of dendrites also. Figure 3 is the dopa reaction of normal negro skin from the breast. The dopa-positive cells are more numerous than in the normal Caucasian skin of Figure 2. In this negro skin most of the dopa-positive cells are round and devoid of dendrites, negativing the common belief that a melanoblast must be dendritic. Figure 4 shows circumanal skin from another negro. The dopa-positive cells are numerous; many of them are dendritic. Figure 5 shows the increased number of dopa-positive cells in the epidermis of a dark brown patch of von Recklinghausen's disease in Caucasian skin. All of these melanoblasts are round, without dendrites. Figure 6 shows Caucasian skin that has been tanned by exposure to X-ray. Here melanin production is very active. The dopa-positive cells of the epidermis are numerous. The many leucocytes in the corium are an expression of the X-ray dermatitis.

Thus, it matters not whether the pigmentation be racial (negro), or pathologically congenital (von Recklinghausen) or acquired, or a reaction to radiation from without (sunlight, radium, thorium, X-ray), the increased production of melanin is attended by an increase in the dopa-positive cells.

Figure 7 shows the dopa reaction of a pigmented mole, illustrating Bloch's conception of the distinction to be made between active and latent melanoblasts. This distinction will be discussed under the caption Latent Melanoblasts. In the upper zone of this mole there is abundant melanin; here the nevus cells are strongly dopa-positive. Passing downward there is less and less melanin and correspondingly the dopa reaction grows fainter and soon disappears. Three-fourths of the cells in this nevus are producing no visible melanin; these cells are dopa-negative. The limitation of melanin production to the upper layers of a pigmented mole is common. It is seen again in Figure 8. In both of these moles the melanin-producing cells are round and devoid of dendrites.

Dendritic Cells

The common belief that melanoblasts must be dendritic is an error carried over from the frog histology which has long dominated human melanogenesis. It is true that active melanoblasts are often dendritic, but they are not necessarily so. In the pigmented skins

and in the moles illustrated, and in malignant melanomas, the active melanoblasts may be of any shape — round, cuboidal, stellate or dendritic. The shape of the cell has absolutely nothing to do with its melanin-producing power or with its malignancy. Figure 9 shows the dopa reaction of another pigmented mole removed from the arm of a negress. This unusual mole consists exclusively of dendritic cells, all of them dopa-positive. The clinical history of such moles differs in no way from that of moles composed of round or other non-dendritic cells.

Melanin Absent

Having become convinced that an increase in the number of dopa-positive cells goes hand in hand with increased activity of pigment formation, we studied the opposite condition — decrease and absence of melanin. Figure 10 shows the dopa reaction from the edge of a patch of vitiligo of negro skin. On the left is seen normal pigmented negro epidermis with its dopa-positive cells. Then comes a zone of bizarre dendritic cells, as is usual at the margin of pigmentation or depigmentation. On the right the colorless skin begins. Here the dopa-positive cells have disappeared, together with the melanin. This disappearance of the dopa-positive cells in vitiligo was described first by Bloch,[24] who reported a similar disappearance of dopa-positive cells from the hair matrix of graying hair.[25]

Figure 11 shows the dopa reaction from the edge of a black spot on the white ear of a guinea-pig. Melanin and dopa-positive cells are abundant in the black spot, totally absent from the white skin. Bloch [7] and his school have shown repeatedly that there are no dopa-positive cells in albino skin and that no amount of radiation will elicit either melanin or dopa-positive cells in such skin, although in non-albino skin it is a simple matter to produce both of them by radiation (Lutz [26]). Thus, whether the absence of melanin be congenital (albino) or acquired (vitiligo), where there is no melanin there are no dopa-positive cells; or, to put it the other way, where there are no dopa-positive cells no melanin is made.

Melanin Reappears

The final proof that dopa-positive cells are concerned in melanin production is that they are the invariable precursors of melanin's appearance or reappearance. Among dermatologists it is well known

that some patches of vitiligo can be stimulated temporarily to repigmentation by exposure to radiation (Buschke,[27] Buschke and Mulzer,[28] With [29]). In such repigmentation of vitiligo, both Bloch and Kissmeyer [30] have reported that the dopa-positive cells reappear first; melanin follows. In my own observations of the repigmentation of scars, this sequence is invariable; it is especially striking in negro skin. Bloch [7, 25] has described the same sequence in the human embryo, Miescher [23] in the eye of the embryo chick, rabbit and guinea-pig, Peck [19] in tanning of human skin by thorium radiation. The dopa-positive cells appear first, melanin next, never in the reverse order. The inference is that the dopa-positive cells are essential to the production of melanin.

The Acanthoses

In his early experiments Bloch soon found dopa-positive dendritic cells to be abundant among the epithelium of the acanthoses. The word is Unna's; it applies to the thickening of the prickle-cell layer of the epidermis seen in psoriasis, papilloma, condyloma and similar lesions. Usually there is no excessive pigmentation, often no melanin whatever. This observation has been confirmed by all dopa workers. I have seen it repeatedly. In fact, the massing of dopa-positive dendritic cells a little distance back from the margin of a granulating wound might be ascribed to the acanthosis always present in this zone quite as justly as to their being forerunners of pigmentation.

According to Kyrle,[31] that rare spirit too early lost to dermatopathology, the cells of the epidermal basal layer have two functions, melanin formation and proliferation. It seems that stimulation of either function increases the number and complexity of the dopa-positive cells. I have been unable to correlate this acanthotic increase of these cells with their pigment function. This phase of their activity awaits adequate explanation.

Mucous Membranes

Among mucous membranes, production of melanin is confined to those of ectodermal origin. Adachi [32] found melanin in the epithelium of the mucous membrane of the cheek, lower lip, prepuce and vagina. He was limited to hand-cut, unstained sections. Using both the silver and the dopa reaction, Redslob [33] found melanin and dopa-positive cells in normal human conjunctiva, Ramel [34] in normal hu-

man "buccal mucosa" (site not stated). Becker [16] found both melanin and dopa-positive cells in Adachi's locations and added the normal mucous membrane from the middle of the cheek and from the pharynx at the level of the hyoid bone. Laidlaw and Cahn [35] found dopa-positive cells and melanin in normal human gum (Fig. 12); Laidlaw (unpublished) in normal anal canal. The presence of melanoblasts explains the occurrence of primary melanoma in these mucous membranes and also the absence of primary melanoma in non-ectodermal mucous membranes which normally harbor no melanoblasts and make no melanin.

In blonds, melanin and dopa-positive cells of the mucous membranes are scanty, as they are in blond skin; in the mucous membranes of negroes and brunets, they are abundant, an observation already made by Adachi in regard to melanin. To the naked eye, such a mucous membrane may look pink and without a trace of pigmentation; nevertheless, on microscopic examination melanin will be found in abundance. Ramel noted racial pigmentation of the mouth in Tziganes; Cahn observed similar racial pigmentation of the mouth in negroes and in a negroid type of Bavarian Jew.

Chromatophores of the Derma

In the upper layers of the derma of every normally pigmented skin there are seen cells of various shapes containing melanin. These cells never give the dopa reaction. Consequently according to the dopa hypothesis they contain no oxidase and they cannot have produced the melanin which they contain. Often these cells are obviously phagocytic; they seize and retain any pigment that happens to enter the skin, such as tattoo pigment, blood pigment and gunpowder. Miescher [36] proved their phagocytic power for melanin and their long retention of it by injecting melanin into living human skin and excising bits of skin at various intervals afterward. To distinguish them from melanoblasts, these cells are called pigment carriers, chromatophores.

Within certain limitations, to be mentioned presently, the dopa reaction is the one reliable method of distinguishing the melanoblast from the chromatophore. This distinction may be seen in any negro skin or pigmented Caucasian skin. Miescher [37] and von Albertini and Walthard [38] have used the dopa reaction to identify melanoblasts in the metastases of malignant melanoma.

Latent (Dopa-negative) Melanoblasts

The use of the dopa reaction as a specific stain for melanoblasts stumbles over the difficulty that the cell capable of producing melanin is not always and everywhere dopa-positive; it does not always contain the oxidase. This is seen readily in pigmented moles and in melanomas where broad areas of the tumor are free from visible melanin and from dopa-positive cells. Here a negative reaction means nothing; only the positive cells count. (The situation is different with the chromatophores of ordinary pigmented skin. Thousands of microscopic sections examined by competent dopa workers have never once revealed a positive dopa reaction in the chromatophores of the upper derma. We may accept them as permanently lacking the melanin-producing oxidase).

In his first publication, Bloch [1] wrote that in malignant melanoma the cells around the growing margin of the tumor were most apt to be dopa-positive, while many of the cells in the center of the tumor were negative — an observation confirmed by Miescher [39] and by von Albertini and Walthard.[38] Both Bloch and Miescher point out that the dopa-positive melanoblast, whether dendritic or non-dendritic, does not always contain melanin, neither is the melanin-containing cell always dopa-positive. These discrepancies are explained best by Bloch's ferment hypothesis, according to which a distinction must be made between the oxidase (which carries out the dopa reaction), and the finished melanin (which does not carry out the reaction). The dopa-positive factor — the oxidase — appears in the cell some time before the resulting melanin is visible (see caption Melanin Reappears); the melanin itself may remain in the cell long after the oxidase has disappeared (Miescher's experimental injection of melanin into human skin).

My own experience is in strict harmony with the ferment hypothesis; but, whatever the explanation, there remains the fact that the dopa reaction must be used with this precaution, that not all cells capable of producing melanin are at all times dopa-positive. In her studies of pigmentation of the skin of the embryo and newly born gray mouse, Steiner-Wourlisch [40] found that even in this normal, progressive pigmentation the production of melanin is not continuous, and the dopa reaction is not continuously present; there are intervals of rest.

Mongol Cells

These ribbon-like cells buried deep in the corium over the sacrum and along the backs of infants of all races are true melanoblasts, the only melanoblasts of mesodermal origin in normal human skin. Bloch [7] and Bahrawy [41] found them to be dopa-positive. As an anomaly I saw them in the neurofibromatous skin of von Recklinghausen's disease from over the sacrum of a Caucasian girl 18 years of age. As shown in Figure 13, they were dopa-positive. They blackened with silver also, proving their scanty pigment to be melanin. Melanomas arising from these cells and from their analogues, the cells of blue nevi, are the only melanotic tumors of human skin to which the name melanosarcoma can justly be applied, a view first formulated by Darier,[42] prompted by a suggestion from Bloch.

Not every nevus that looks bluish black conforms histologically to the Tièche-Jadassohn blue nevus.[43] Even epidermal melanin in the tips of long rete pegs will look bluish and not brown if there is little or no melanin in the surface epithelium (Fig. 12). Sato [44] has made a similar observation of ordinary nevus nests that were deeply placed in the corium. The diagnosis of blue nevus, and the sequent melanosarcoma, should rest on microscopic examination.

Sarcoma and Carcinoma

The dopa reaction has no relation to malignancy, as such. In repeated tests of various forms of non-melanotic sarcoma and carcinoma, I have found the tumor cells to be consistently dopa-negative.

Epithelioma

Recalling Kyrle's dictum that the function of the epidermal basal cells is twofold, proliferation and melanin production, it might be expected that the progeny of these cells in forming an epithelioma would show some melanoblastic characteristics, especially since acanthosis or thickening of the prickle-cell layer is attended by an increase in the dopa-positive cells. This expectation is fulfilled. As in all tumors, function is performed imperfectly and irregularly. In some epitheliomas I find no dopa-positive cells; the dopa-positive cells of the overlying skin stop some distance back from the edge of the ulcer as they do in non-malignant granulating wounds. In other

epitheliomas the dopa-positive cells continue in the surface epithelium over the tumor, or a few scattered dopa-positive cells are seen among the tumor epithelia, chiefly in the basal layer. As in the acanthoses, these cells are mostly dendritic. Their presence in an epithelioma seems to have no significance.

Melanosis Coli

In melanosis coli the tunica propria of the mucosa contains many large, round, stellate and spindle cells loaded with yellow-brown granules. In the specimens which I have examined the pigment reacts like melanin in that the fine granules blacken quickly in silver, while the larger granules require a long time, perhaps several days. Current opinion is divided as to the nature of the pigment, but is agreed that it has been absorbed from the intestinal contents and phagocytized by these cells. The view that the cells are phagocytes is corroborated by Walthard's [45] finding them dopa-negative at autopsy.

Negative dopa reactions of autopsy material are open to suspicion, owing to the length of time that necessarily elapses between death and the immersion of the tissue in dopa. By the kindness of Doctor Janssen, I was able to immerse a specimen of diffuse melanosis of the upper part of the rectum in dopa within two hours of its excision from the living body. Microscopically the mucosa presented the typical appearance of melanosis coli. The pigment-bearing cells were dopa-negative. There were no dopa-positive cells, except polynuclear leucocytes which abound in this mucosa. I have treated many fresh surgical specimens of colon and rectum with dopa and also tested them for melanin with silver with consistent negative results. The conclusion is that there are no melanoblasts and no melanin in the colon or in the rectum above the mucocutaneous junction. It follows that the occurrence of primary melanoma above this line is highly improbable. If it occurs, it must spring from misplaced islands of ectoderm.

The Silver Controversy

Soon after Bloch's first publications Heudorfer [46] declared that there is nothing specific in the dopa reaction and that it merely duplicates pigment staining with silver, an assertion endorsed by Lem-

mel [47] and Meirowsky.[48] My own experience, covering hundreds of sections of skin stained with various silver techniques, and additional hundreds of dopa sections compared with silver staining of the same skin, agrees absolutely with Bloch's statement that the dopa reaction and the silver reaction are totally different things. To an experienced dopa worker it is obvious that Bloch's critics have been misled by overstained dopa sections. The correct reactions could never be mistaken for one another. Silver blackens melanin wherever found, in the derma as in the epidermis, in melanoblasts, phagocytes, and free in the lymph spaces indifferently. If the dopa-positive cells happen to contain melanin they will stain with silver; otherwise not. Dopa, on the other hand, singles out the active melanoblasts, leaving the melanin in the resting cells unstained. The only melanin that blackens with dopa is the melanin inside of a dopa-positive cell, on which the dopa-melanin is adsorbed or deposited.

The Dimethylparaphenylendiamin Controversy

On the publication of the dopa reaction Kreibich [49] declared that he had long secured similar effects from dimethylparaphenylendiamin (one of the components of the Schultze-Winkler formula), a position in which he is supported by Meirowsky,[48] who goes even further and finds the dopa reaction duplicated by a whole series of easily oxidizable phenols. After many trials with this most highly praised phenol of this group, I agree with Bloch [12] and with Walthard [45] that this phenol, like silver, stains only the melanin and not the protoplasm of the cell. Even when the reaction succeeds, the demonstration of the dendritic cells is far inferior to that of the dopa reaction, as Meirowsky himself admits.

The Mast Cell Controversy

Of the many controversies prompted by the dopa reaction, the oddest and the least necessary would seem to be the difference of opinion of equally well qualified observers over the reaction of mast cells. That master of dermatopathology, P. G. Unna, wrote long ago that mast cells abound in the snout of the white rat and in human neurofibroma. It is a simple matter to immerse fresh frozen sections of these tissues in dopa solution and counterstain them with cresyl

violet. In such sections it is seen beyond any possible doubt that Bloch scores once again. The mast cell, both in the white rat and in human neurofibroma, is dopa-negative. The reader may see this for himself in Figures 5 and 13, from the same von Recklinghausen neurofibroma. These sections contain myriads of mast cells, not one of which has become visible in the dopa solution.

Summary

1. Bloch's dopa doctrine is endorsed as the best working hypothesis of melanin production in human skin.

2. The dopa reaction is indispensable in the study of pigmented moles, melanoma and the movements of melanin.

3. In the identification of melanoblasts with the dopa reaction, only the positive cells are significant.

4. The appearance of dopa-positive dendritic cells in non-pigmented acanthoses remains unexplained.

5. In the controversies which have arisen over the dopa reaction, Bloch's histological findings are corroborated.

In conclusion I must thank Professor Bloch for his kindness in sending me a portion of his dwindling store of dopa in the beginning of these studies two years ago; Dr. S. N. Blackberg of the Department of Pharmacology, who showed me how simple a matter the dopa reaction could be made to be; Dr. Jerome Webster for many fresh specimens from his plastic surgery clinic; and Professor Purdy Stout who generously places his choicest material at my disposal.

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DESCRIPTION OF PLATES

PLATE 83

All figures are untouched photomicrographs of dopa reactions at pH 7.4 and at 37°C for from 3 to 4 hours.

FIG. 1. Hodgkin's lymph node. Myelogenous leucocytes black; lymphocytes and collagen colorless.

FIG. 2. Normal Caucasian skin from breast. In the basal layer of the epidermis there are blackened melanoblasts of various shapes, both round and dendritic. They are few in number and spaced far apart. The rest of the epithelium and the collagen are colorless.

FIG. 3. Normal negro skin from breast. In the epidermis the dopa-positive cells (melanoblasts) are more numerous than in Fig. 2. Almost all of them are round, not dendritic. The chromatophores of the corium are visible as pale gray spindle cells. They contain no ferment and do not blacken in dopa.

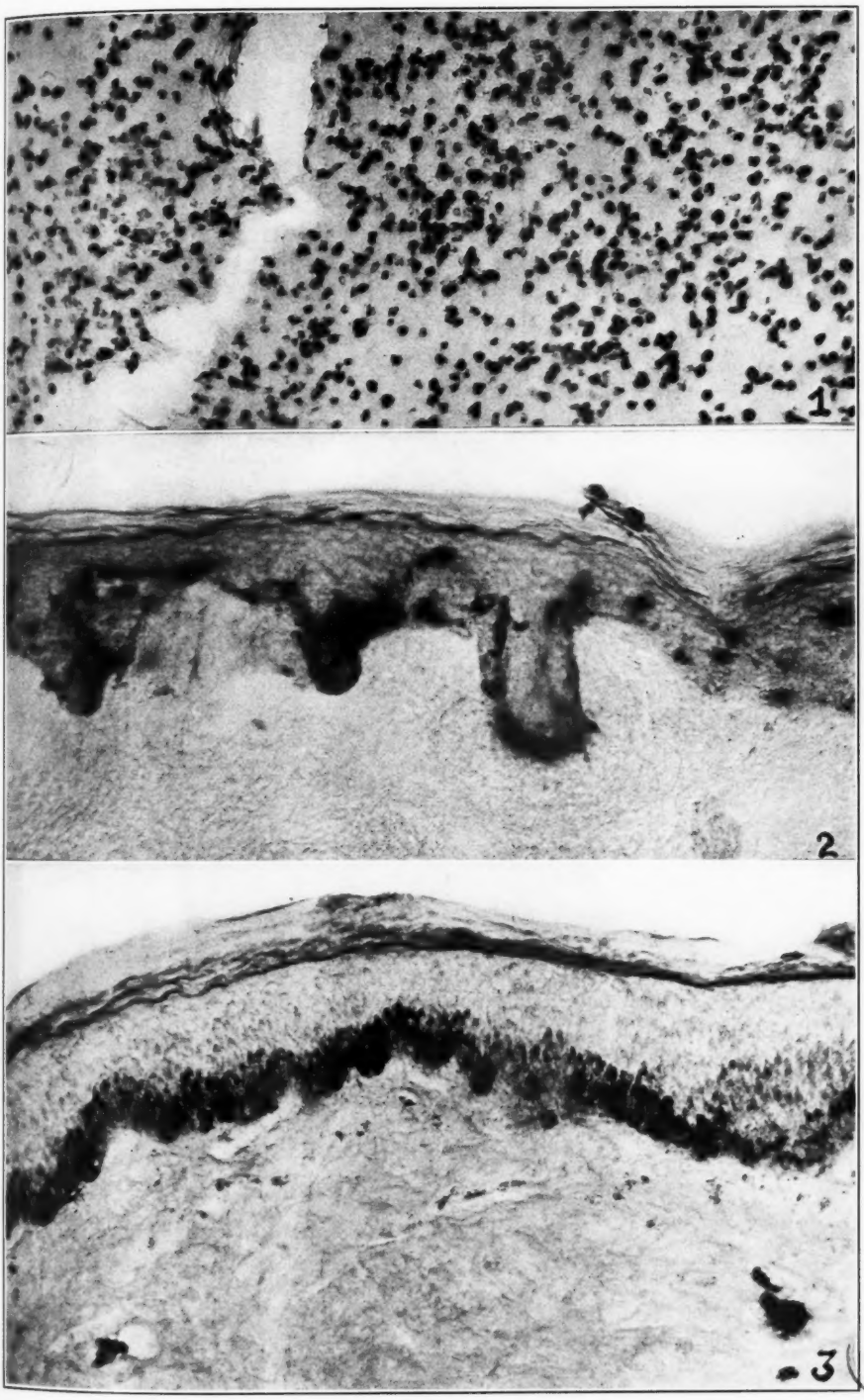
1
2
3

PLATE 84

FIG. 4. Normal circumanal skin from another negro. The dopa-positive cells of the epidermis are very black, very numerous and most of them are dendritic. In this corium there are many chromatophores loaded with melanin; none of them blacken in dopa.

FIG. 5. Dark brown patch of von Recklinghausen pigmentation of Caucasian skin. Melanoblasts of the epidermis very numerous. Almost all of them are round, a few are dendritic.

FIG. 6. Caucasian skin tanned by X-ray. Dopa-positive cells of the epidermis much more numerous than in the normal epidermis of Fig. 2. In the papillary layer of the corium there are many black leucocytes, an expression of the X-ray dermatitis.

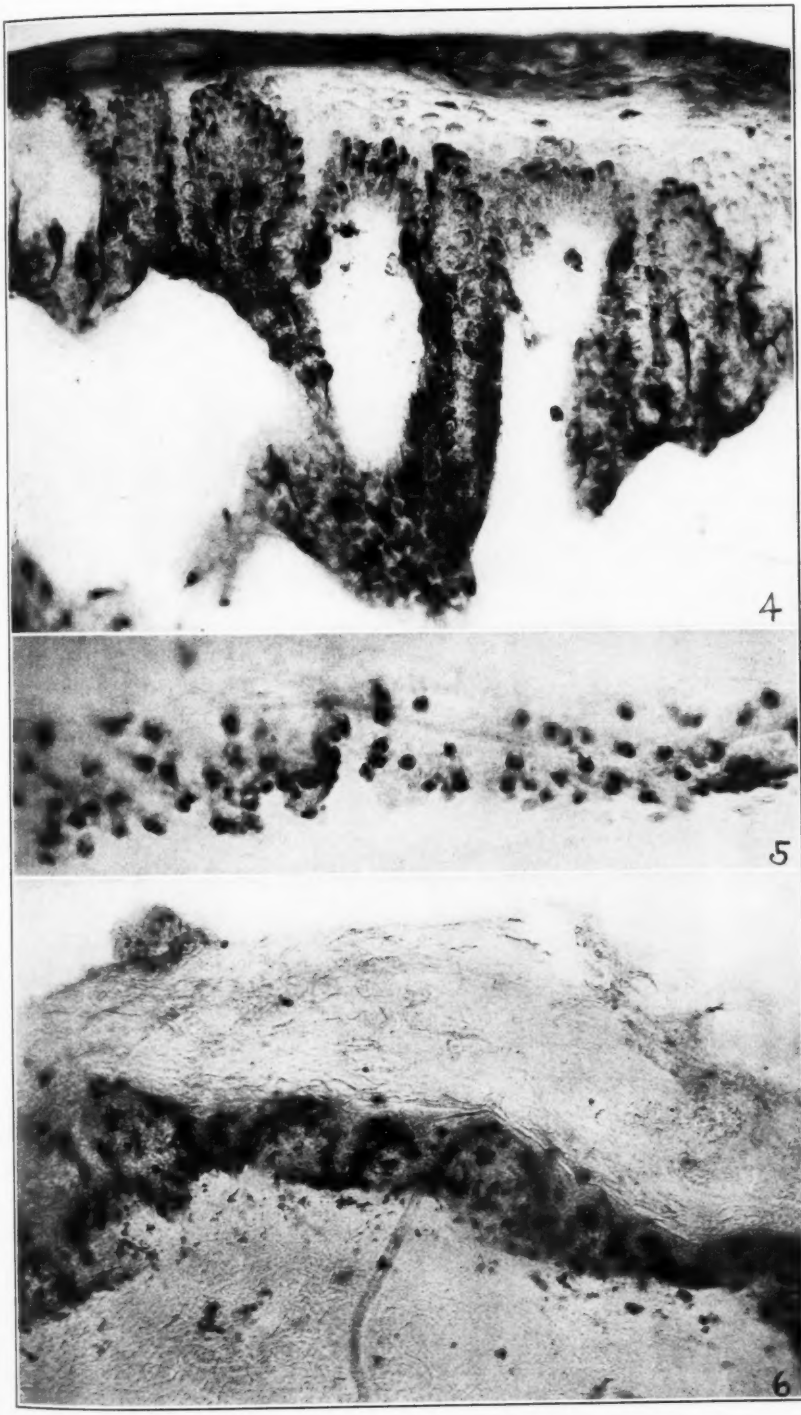
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PLATE 85

FIG. 7. Pigmented mole. In the center is a hair in its follicle. There is much melanin in the upper layers of the mole and here the nevus cells are strongly dopa-positive, very black. Passing downward the melanin decreases and correspondingly the dopa reaction becomes fainter. In the lower part of the mole, where no melanin is being produced, the nevus cells are dopa-negative.

FIG. 8. Another pigmented mole with the same features as Fig. 7. In both of these moles the active melanoblasts, the nevus cells, are round and free from dendrites.

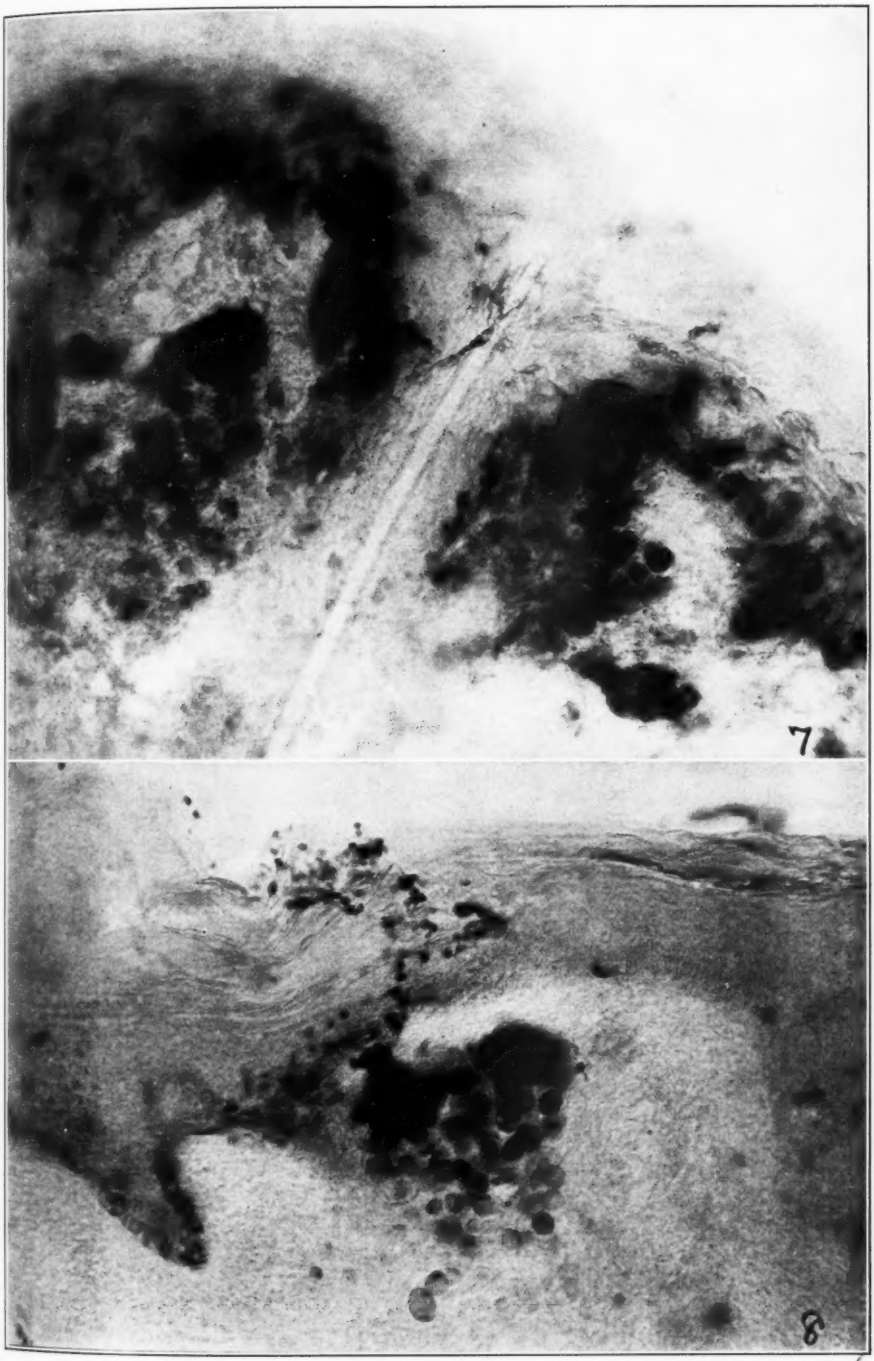

7
8

PLATE 86

FIG. 9. Unusual pigmented mole consisting entirely of dendritic cells, all of them dopa-positive. From the arm of a negress.
(FIGS. 5, 7, 8 and 9 from patients of Dr. Webster.)

FIG. 10. Margin of patch of vitiligo of negro skin. On the left, normal negro epidermis with abundant melanin and dopa-positive melanoblasts. In the middle, the zone of bizarre dendritic cells usually seen at the margin of active pigmentation or depigmentation of the epidermis. On the right, the colorless epidermis; both melanin and dopa-positive cells have disappeared. (Patient of Dr. Marie Karelitz.)

FIG. 11. Edge of black spot on white ear of guinea-pig. In the epidermis of the black spot, melanin and dopa-positive melanoblasts are abundant; they are totally absent from the epidermis of the white skin. At the margin of the black spot, where melanin becomes scanty, the outline of the dendritic melanoblasts is seen more clearly.

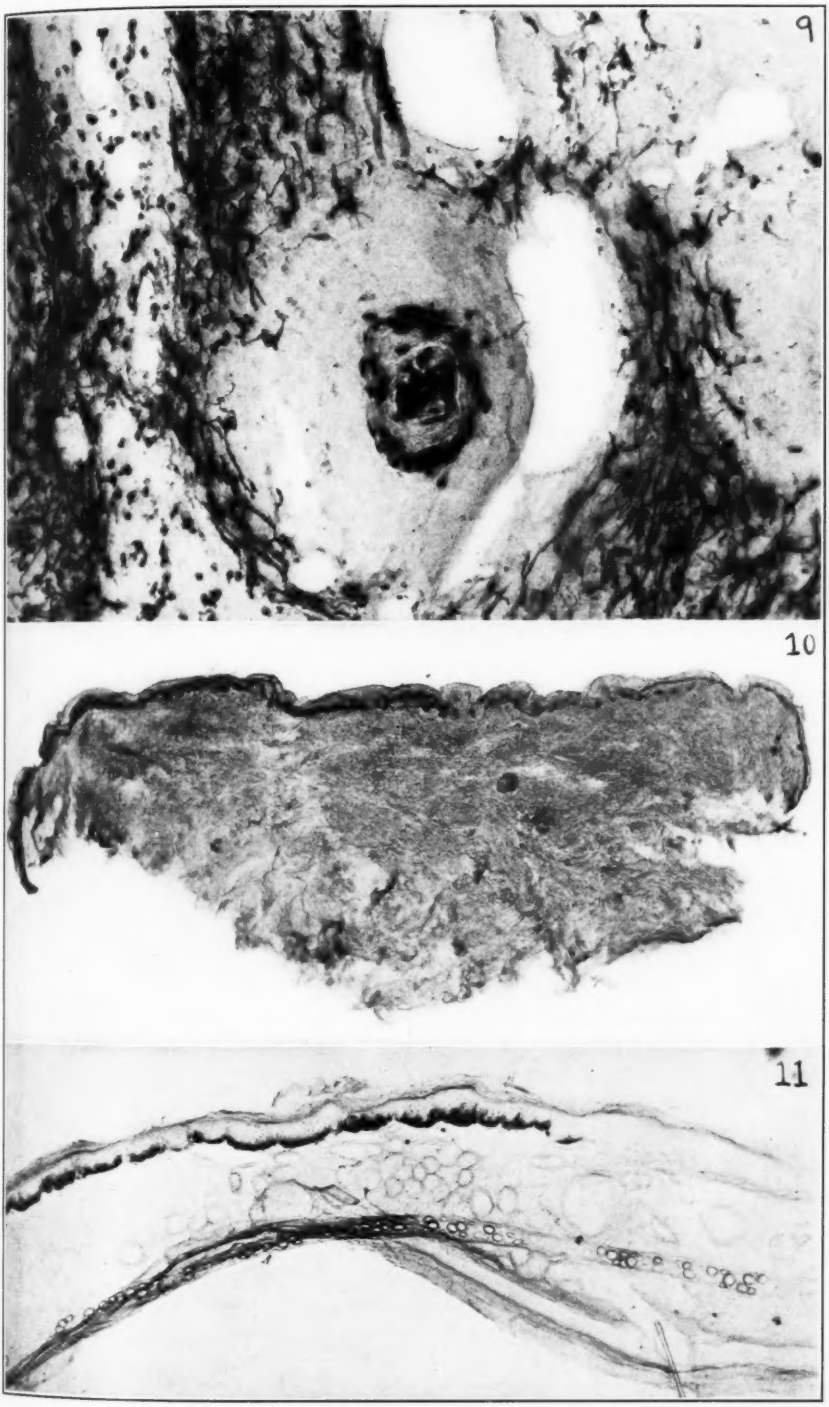
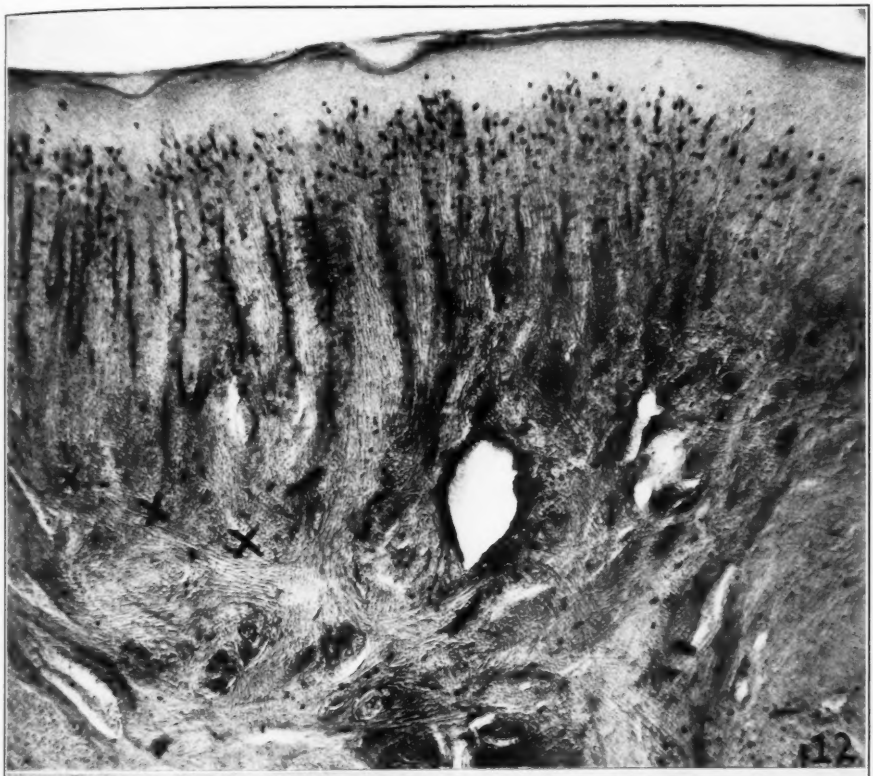
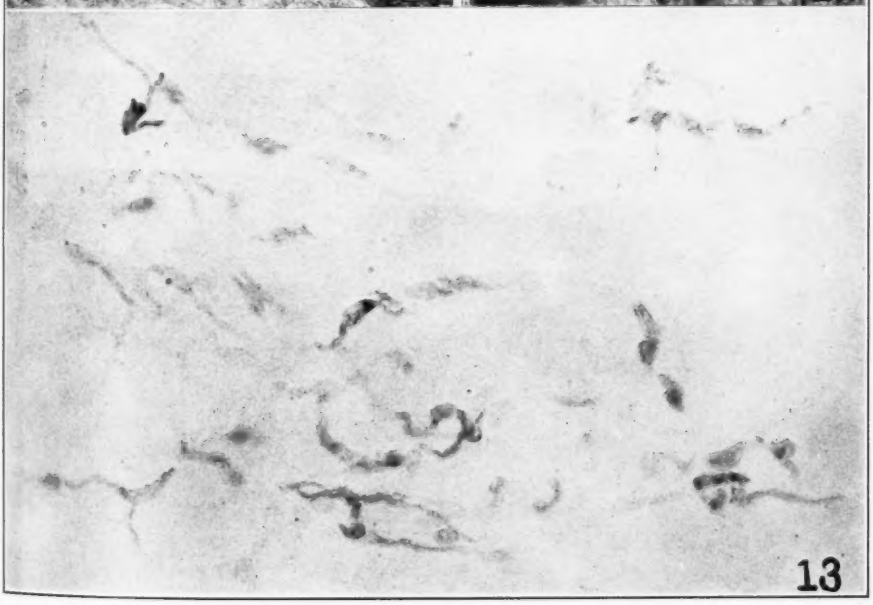


PLATE 87

FIG. 12. Slate-colored pigmentation of gum of Latin-American; black hair, dark eyes. In the middle third of the very thick stratified epithelium there are many dopa-positive dendritic cells. The melanin itself is situated chiefly in the lower end of the very long, slender rete pegs. The deep situation of the melanin and the absence of superficial pigment accounts for the blue color of the gum, although the melanin is exclusively in the epithelium and not in the corium, as in the Tièche-Jadassohn blue nevus. An X mark has been placed just below the tips of the longer rete pegs. (Patient of Dr. Lester Cahn.)

FIG. 13. Dopa-positive Mongol cells. Neurofibromatous skin in von Recklinghausen's disease from over the sacrum of Caucasian girl (Polish Jew) 18 years of age. The corium is very thick, averaging 1.5 cm. The groups of ribbon-like cells are situated deep in the corium, in its middle or lower third. They are filled with fine granules of melanin. (Patient of Dr. Webster.)





Laidlaw Melanoma Studies. I

MELANOMA STUDIES *

II. A SIMPLE TECHNIQUE FOR THE DOPA REACTION

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Bloch's dopa reaction is a specific stain for melanoblasts and for myelogenous leucocytes. These cells are believed to contain an organized ferment which oxidizes dioxyphenylalanin (dopa) to melanin. The dopa-melanin colors the reacting cell black. This blackening of the cell is the dopa reaction.[1]

In the published descriptions success with the dopa reaction is attributed to a somewhat meticulous observance of certain chemical details. Blackberg proved that many of these precautions can be dispensed with. In fact, we have come to treat the dopa reaction with so little ceremony that when a specimen comes into the laboratory late in the day we put frozen sections in a small vial of buffered dopa in an inside pocket until the solution turns sepia brown, wash the sections in tap water and mount them the next day. However, we prefer to work with a uniform temperature and to control the reaction with the microscope. The following simple method gives constant and accurate results. The reagents required are a stock solution of dopa and the Sorensen buffers.

THE STOCK DOPA SOLUTION

This is a 1:1000 solution of 3, 4-dioxyphenylalanin (abbreviated to dopa) † in distilled water. Dopa is a phenol extracted from *Vicia faba*, a common vetch or sow bean. The levorotatory preparation should be used, since Bloch and Schaaf [2] and Peck and coworkers [3] have shown that melanoblasts have little or no oxidizing power over

* Received for publication April 22, 1932.

† When ordering, specify "for Bloch's dopa reaction." Supplied by the American branch of Hoffmann-LaRoche, Nutley, New Jersey, at 95 cents per gram. With the minute quantity used, the cost of staining a dozen or more sections is less than 2 cents.

the dextrorotatory form. In powder, as purchased, dopa keeps indefinitely at room temperature.

Dissolve 0.3 gm. of dopa powder in 300 cc. of cold distilled water. Keep well corked in the refrigerator, where it will remain good for many weeks. The solution is usable as long as it is colorless or only slightly tinged with red. Darker red solutions should be rejected; they oxidize too quickly and overstain the sections.

Correcting an Error

Dr. Peck calls our attention to a printer's error that has dogged the steps of the dopa reaction. Bloch uses dopa in a solution of 0.1 of 1 per cent and he has never used anything else. Unfortunately in the literature, even in Bloch's and Peck's own papers, in Romeis' popular Taschenbuch (p. 295), and in Krause's Enzyklopädie der mikroskopischen Technik (Vol. 3, p. 1785), the concentration has been printed incorrectly as from 1 to 2 per cent. Let it be understood then that the correct proportion is 1:1000 and that any statement to the contrary is a printer's error.

The Buffers

Dissolve 11 gm. of disodium hydrogen phosphate (Na_2HPO_4+ 2 H_2O) in 1000 cc. of distilled water.

Dissolve 9 gm. of potassium dihydrogen phosphate (KH_2PO_4) in 1000 cc. of distilled water. Both of these buffers are kept in the refrigerator.

Just before cutting the sections, buffer to 7.4 by adding 2 cc. of the potassium phosphate and 6 cc. of sodium phosphate buffer to 25 cc. of the stock dopa solution. For a small batch of a dozen sections we use 15 cc. of the buffered solution, but we prepare double the quantity immediately required in order to have enough to renew the solution in half an hour. Return the stock dopa solution and the surplus of the buffered solution to the refrigerator immediately; at room temperature the stock solution soon oxidizes and turns red, the buffered solution tends to turn brown.

At a given temperature, the speed of the reaction is determined by the pH. At 7.4 the reaction will be finished in 4 or 5 hours at 37° C. When in a hurry, we hasten the reaction by using only 1 cc. of the potassium buffer, giving a pH of 7.7; or we may omit the potassium

buffer, obtaining a pH of 8.2. Such solutions react quickly, in about 60 minutes at 37° C. They should be inspected every 20 minutes to forestall overstaining. These hurried reactions are apt to be overstained; the slow reactions give much more delicate pictures.

A trace of acid inhibits the reaction. A trace of alkali hastens it. All glassware therefore should be scrupulously clean.

Fresh Tissue Required

Melanoblasts: For a dopa reaction of melanoblasts the tissue must be fresh. After death or excision of the tissue from the living body, the intracellular ferment soon diffuses into the surrounding tissue and it is quickly destroyed by most fixatives and preservatives. The ideal material is a frozen section of fresh tissue made immediately after excision from the living body. However, it is difficult to cut fresh tissue neatly. In practice we follow Bloch's present custom of hardening thin slices of freshly removed tissue in 5 per cent formalin for 2 to 3 hours. After this short fixation the tissue cuts better and such experienced observers as Bloch, Miescher, Becker and Peck testify that the reaction is in no way impaired by this short stay in 5 per cent formalin. Neither the gross specimen nor the sections should be permitted to lie in water for more than a few seconds. Water and dilute alcohol extract the ferment rapidly.

In our experience, Walthard goes too far in permitting a stay of 3 days in formalin. We have made innumerable efforts to preserve tissue overnight for a dopa reaction the next day. We have tested commercial formalin (plain and neutralized with sodium hydrate), and Merck's neutral formalin, both plain and further neutralized with chalk (Cajal's practice), from 1 per cent to 100 per cent. Melanoblasts last best in 5 per cent formalin, whether neutralized or not. However, the result is always a gamble and depends on the quantity of ferment originally present in the cells. If in the fresh tissue the dopa-positive cells are black and numerous, they may still be found after 3 weeks or more in 5 per cent formalin. If in the fresh tissue the dopa-positive cells are pale, indicating little or feeble ferment, they will disappear in any formalin within 10 to 12 hours. All workers agree that the best concentration of formalin is 5 per cent. Weaker solutions extract the ferment; stronger formalins abolish the reaction quickly.

Refrigeration: We have tried with little success to preserve melanoblasts in the refrigerator. As with formalin, strongly positive melanoblasts survive for several days; faintly reacting cells disappear overnight.

Leucocytes: The ferment of the myelogenous leucocyte endures much longer than that of the melanoblast. In fact, fresh leucocytes react all the better for a few days in strong formalin and after 2 or 3 months they may still react well. Even for leucocytes there is a time limit, 3 to 4 months, beyond which most of them no longer blacken in dopa solutions.

Cutting the Sections

Frozen sections are obligatory since the chemicals of celloidin and paraffin embedding would destroy the ferment. It is important to remember that water extracts the ferment quickly. Neither the block nor the sections should lie in water longer than the few seconds of a quick rinse. Before cutting the sections, the dopa solution should be buffered and poured into dishes ready to receive the sections without delay.

In order to exhibit the long dendrites of melanoblasts some of the sections should be very thick, 75 to 100 microns; others may be from 20 to 30 microns for better detail. Sections of fresh tissue are dropped from the knife directly into dopa. If the tissue has been in formalin, the sections are rinsed for a few seconds in distilled water and placed promptly in the dopa solution. Since the reaction is an oxidation the dish is left uncovered for free access of air.

Temperature and Time

The dish of dopa containing the sections is put in the incubator at 37° C for about half an hour. Then the fluid is replaced by fresh solution, which in the meantime has been kept cold in the refrigerator. For this renewal of the solution there is a reason. Some tissues are sufficiently acid to lower the pH below 7.0, in which event the fluid remains red and the cells do not oxidize dopa to melanin. They remain colorless. On the other hand, tissue that has been in formalin, especially neutralized formalin, hastens the oxidation, darkens the fluid prematurely and easily overstains. Since it is impossible to foresee the presence of these disturbing factors, and since water

cannot be used to wash them out, we make it a routine practice to change the dopa. The first dopa washes out any objectionable substances and the reaction proceeds unhindered in the fresh solution. At times we have found sections of rectum and colon so acid that two changes of dopa were required before the red of the acid solution changed to the sepia brown of a correct reaction. Under these circumstances a liberal quantity of dopa solution should be used.

Having replaced the first dopa with fresh solution, the reaction is inspected every half hour. In 2 or 3 hours the fluid turns reddish, then sepia brown. The appearance of the sepia tint signals the end of the reaction. At this point a section is rinsed and examined under the microscope. In the perfect reaction the bodies of the dopa-positive cells (melanoblasts and leucocytes) are gray or black, melanin retains its natural yellowish brown color, and collagen is colorless or the palest shade of gray. If a darker stain of melanoblasts is desired, the section is returned to the dopa solution for another half hour or so. The beginner will do well to mount a section every half hour from the beginning to the end of the reaction, continuing until the solution has become black. Such a series of sections is an instructive panorama of the progress of the reaction. It will show him clearly that much of the criticism of the dopa reaction is based on overstained sections.

The time in the incubator will vary with different specimens. Of two tissues prepared alike, cut and dropped into separate dishes of dopa at the same time, one may darken more quickly than the other. The color of the section is a fair guide to the progress of the stain. A well stained section is colorless or pale gray; a pronounced smoke gray indicates overstaining. However, it is much better practice to control the reaction with the microscope.

Bloch, Walthard and European writers generally prefer a slow reaction at a lower temperature, leaving the sections in dopa for from 12 to 24 hours "at room temperature," which they state to be 18° C. In an American laboratory it is difficult to secure a constant temperature of 18° C (64° F). Our own laboratory is 23° C (73° F) in winter and from 26 to 28° C in summer. Sections left in dopa overnight are invariably found to be overstained in the morning. For this reason we use 37° C as more easily controlled.

The reaction proceeds much more quickly in the paraffin oven at 56° C. At this temperature the sections should be inspected more

frequently to prevent overstaining. The objection to rapid reactions at high temperatures is that the fluid soon darkens from spontaneous oxidation of dopa to melanin. The dopa-melanin stains the whole section an even dark brown. In these rapid reactions it is not easy to seize the exact point where the reaction should be checked to prevent overstaining. Bloch is certainly right in insisting that the slower reactions give the more delicate pictures.

Counterstaining and Mounting

The reaction finished, wash the sections in water, dehydrate, clear and mount in balsam, or counterstain in any way desired. The dopa stain is a fast black which resists all the usual reagents except hydrogen dioxide and similar oxidizing bleaches. The paradox of melanin being produced by the oxidation of dopa, and disappearing with further oxidation, is explained by its being the one colored stage in a series of oxidations, the stages before and after it being quite colorless.

The browns, blacks and grays of a correct dopa reaction form an extremely delicate picture. We dislike to obscure it with a counterstain. A dopa section, a silver stained section and a wholly unstained section mounted side by side constitute a very instructive series. Some sections of the batch may be counterstained for general topography or for special features, such as mast cells, plasma cells, or elastic fibers. As a counterstain Bloch and dermatologists generally use methyl green-pyronin. We prefer cresyl violet well differentiated with alcohol, as giving a paler ground. All counterstains take better if the dopa sections are first dehydrated, cleared, and brought back through alcohol to water.

Surgical Preparation of the Skin

Through all dopa literature runs the warning that surgical preparation of the skin with chemicals, especially with iodin, inhibits the dopa reaction. If true, this would be unfortunate; for most of the skin coming into a surgical laboratory has been painted with iodin. However, the statement cannot be strictly true because the greater part of our collection, including some of our finest specimens of dopa-positive dendritic cells, consists of skin that was painted with iodin and washed with alcohol in the usual surgical way.

In order to test this point, in the excision of a series of scars and pigmented moles Dr. Jerome Webster kindly offered to use no skin preparation other than washing with soap and water followed by alcohol. These specimens were sectioned and placed in dopa within an hour of their excision, without contact with formalin or even with water, the sections being dropped from the knife directly into dopa solution. The results were very fine but we cannot say that they were uniformly better than in many similar specimens that had been painted with iodin and immersed in dopa with equal promptitude. As already noted in the efforts to preserve melanoblasts in formalin and with refrigeration, it is possible that strongly positive cells survive the iodin, while weakly positive cells disappear. Be that as it may, our experience indicates that the prospective investigator of the dopa reaction need not be deterred from using surgical material, even though it has been treated with iodin. We have even had good reactions from skin and mucous membranes that had been painted with picric acid, as used in the Squier Urological Clinic, to which we are indebted for some very fine specimens.

Leucocytes

Myelogenous leucocytes stain more quickly than melanoblasts. To secure a delicate picture of leucocytic granules, the reaction must be checked before the melanoblasts are fully stained. As in the Schultze-Winkler reaction, leucocytes stain more uniformly in a strongly alkaline solution. Taking advantage of this principle, Bloch and Peck [4] have recently recommended a special dopa technique for myelogenous leucocytes in blood films; it is useful for sections also.

The films are fixed in hot formol fumes for 20 minutes and immersed in 1:1000 dopa prepared with physiological salt solution. Then 0.2 cc. of 0.1 normal sodium hydrate is added for each 10 cc. of the stock dopa. The mixture turns yellow, then brown. It is left at room temperature for from 1 to 2 hours. A slide is examined microscopically every half hour. When the leucocytic granules are stained uniformly, usually in 1 to 1½ hours, the slides are washed in running water and treated like ordinary films.

Bloch and Peck have devised an ingenious method of accentuating the sharpness of the granule staining by washing the film in distilled water and immersing it in 2 per cent silver nitrate for 2 hours. Wash

again in distilled water; leave 10 minutes in saturated solution of hypo, wash in distilled water, stain the nuclei with hematoxylin and mount in balsam.

SUMMARY

A simplified technique for the dopa reaction is described and discussed in detail.

NOTE: For the interpretation of the dopa reaction with illustrations and complete literature, the reader is referred to the previous paper.[5]

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A STUDY OF THE REPAIR OF ARTICULAR CARTILAGE AND THE REACTION OF NORMAL JOINTS OF ADULT DOGS TO SURGICALLY CREATED DEFECTS OF ARTICULAR CARTILAGE, "JOINT MICE" AND PATELLAR DISPLACEMENT *

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A review of the literature reveals that opinion is divided as to whether or not articular cartilage is capable of regeneration. Certain workers have reported having observed regeneration of articular cartilage, yet the explanations offered regarding the manner of this regeneration are not in agreement. Because of these conflicting reports it was decided to study surgically created defects of articular cartilage in order to determine not only the ability of articular cartilage to regenerate and its manner of regeneration, but also whether or not any constant intra-articular changes could be ascribed to the existing articular cartilage defect. In certain experiments a portion of the removed cartilage was replaced in the joint from which it was removed in order to note its fate and to see if its presence resulted in any pathological changes. In a few experiments marked joint changes were associated with accidental displacement of the patella. These are reported because they seem to be of value in explaining similar changes noted by previous workers.

MATERIALS AND METHODS

The knee joints of normal, young adult dogs were used in all experiments. Each experimental procedure was carried out upon at least four joints so as to obtain for study similar lesions of four, twelve, twenty and twenty-eight weeks duration.

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In the first group of joints a thin strip of cartilage was removed from the weight-bearing surface of the medial femoral condyle and from the middle of the patellar groove. In a second group of joints a single thin fragment of articular cartilage was removed from the patellar groove, one-half of the fragment being replaced as a loose body or cartilage "joint mouse." From the third series of joints a strip of articular cartilage and subchondral bone was removed from the concavity of the patellar groove, divided, and one fragment was returned to the joint cavity as a cartilage and bone "joint mouse." In two dogs disarticulation through one knee joint was done. In these instances the synovial membranes and joint capsules were sutured over the exposed femoral articular surfaces. All operations were performed aseptically under ether anesthesia. Postoperatively the dogs were allowed the freedom of an indoor stall and an outdoor pen. The joints operated upon were not immobilized or splinted.

After varying periods of time each dog was etherized and the blood vessels of the rear extremities were perfused with 6 per cent acacia-saline solution. The perfusion was terminated by the injection of a suspension of graphite. This procedure was carried out so as to fill as many of the blood vessels and capillaries as possible with a substance that could easily be recognized both on macroscopic and microscopic examination. A 6 per cent acacia solution made up in 0.85 per cent sodium chloride solution was used as the perfusate. The graphite suspension was prepared from Hydrokollag 300, as described by Drinker and Churchill.[1] The suspension was repeatedly centrifuged until aggregates large enough to cause embolism were reduced to a minimum.

The injection of the blood vessels of the rear extremities was accomplished by means of a perfusion pump.* The following method was used. Each dog was anesthetized with ether followed by sodium veronal intravenously 25 to 35 mg. per Kg. A midline abdominal incision was made and the large vessels of the abdomen and pelvis were exposed. Loose ligatures were passed around the lower abdominal aorta and at the same level around the inferior vena cava. The sacral artery was then freed of all its branches and a wash-out cannula directed toward the aorta was inserted. The pump was ad-

* For the use of this apparatus we are indebted to Dr. C. K. Drinker. The method of operating this pump is described by Drinker, C. K., Drinker, K. R., and Lund, C. C., *Am. J. Physiol.*, 1922, **62**, 1–92.

justed so as to deliver 220 to 260 cc. per minute of the warm (37° C) perfusate at a pressure which was approximately the same as the blood pressure of the dog. As the perfusion was begun the ligature about the aorta was tied and the right side of the heart was opened. In this manner a circulation of 6 per cent acacia was substituted instantaneously for the normal circulation of the rear extremities. Perfusion was continued until all grossly detectable blood had been washed out. At that time 100 cc. of prepared graphite suspension was forced into the cannula through the wash-out opening by means of a 100 cc. syringe. Enough pressure was used to maintain a short column of graphite ahead of the perfusing fluid. As the last of the graphite was injected the sacral artery and inferior vena cava were ligated so as to prevent its escape. The rear limbs were then skinned, amputated and before immersion in 10 per cent formaldehyde solution the muscle bellies were separated sufficiently to allow ready penetration of the fixative. After the legs had become well hardened, the soft tissues were completely removed. The joints were opened, examined and photographed. Numerous blocks of tissue from the articular surface, underlying bone and synovial membrane were taken for microscopic study. The blocks of tissue containing bone were decalcified in 5 per cent nitric acid solution and embedded in celloidin. The blocks of synovial membrane were for the most part embedded in paraffin. Microscopic sections were stained routinely with hematoxylin and eosin. Occasional sections were stained by special methods for the demonstration of fibrin and collagen.

Experiment I. Repair of Defects in the Hyaline Cartilage of the Weight-bearing and Non-weight-bearing Articular Surfaces, and the Reaction of Joints to Displaced Patellae

Operation: Each knee joint was opened by a longitudinal incision just lateral to the patella. Bleeding into the joint space was carefully avoided. The patella was displaced medially and the joint was sharply flexed so as to expose the weight-bearing articular surface of the medial condyle. A small, thin piece of cartilage which averaged 4.6 by 3 by 0.5 mm. in size was removed from this area by means of a gouge. In each joint another thin strip of cartilage averaging 10 by 3.3 by 0.5 mm. in size was removed in the longitudinal axis from the

depth of the patellar groove. The patella was replaced and the incision was closed in layers by continuous sutures. The skin was approximated with interrupted mattress sutures of silk and a collodion dressing applied.

The fragments of cartilage removed were measured and placed in Zenker's fluid for fixation. Subsequent histological sections showed all of these fragments of cartilage to be entirely normal. In two of the lesions the calcified zone of cartilage had been removed in small areas, together with the overlying articular cartilage. Such traumatization was apparent at operation because of slight oozing of blood from the injured subchondral blood vessels. Postoperatively, a small effusion occurred in three of the joints. In these joints, and two others showing an increase in synovial fluid, the patellae were found displaced to the medial aspect of the femoral articular surface. Such patellar displacement is worthy of emphasis since it appeared to be the important factor of sterile irritation which resulted in extensive intra-articular pathology, whereas in the joints in which the patellae remained in their normal positions, the changes observed were very slight, or absent.

Macroscopic Examination of Joints

The knee joints containing defects in articular cartilage of four, twelve and twenty weeks duration were all found to contain an excess (2 to 5 cc.) of synovial fluid which was viscid and light amber in color. Differential cell counts made on these fluids immediately postmortem showed an average of 62 per cent mononuclear phagocytes, 19 per cent lymphocytes, 17 per cent polymorphonuclear leucocytes and 2 per cent synovial cells. The patella in each instance was displaced so that it rested upon the inner side of the medial patellar ridge of the femur. The patellae showed varying degrees of atrophy and degeneration most marked in the joints with lesions of four and twelve weeks duration. In the joints least involved the cartilage had disappeared in an area of about 5 mm. in diameter, leaving subchondral bone exposed. In the most markedly altered joint the patella showed practically complete degeneration of cartilage with roughening and fragmentation of the underlying bone.

The synovial membrane in these joints showed marked villous overgrowth in the more vascular portions. On microscopic examination these villi were seen to consist of vascular connective tissue

centers, surrounded in each instance by a number of layers of synovial lining cells. With few exceptions such villi were but moderately infiltrated with lymphocytes and mononuclear phagocytes. There was no exudate or cellular reaction in any of the joints of a degree suggestive of bacterial infection. Another manifestation of these joint changes was that some form of pannus had grown out from the synovial membrane at the margin of the articular cartilage. Such pannus was made prominent by the intra-vascular graphite injection (Figs. 1 and 2). The defect in the patellar groove of twelve weeks duration (Fig. 2), was covered in its upper one-half by the downgrowth of blood vessels and connective tissue from the upper margin of articular cartilage. The blood vessels and capillaries were very numerous and well filled with graphite (Fig. 3). In the joint containing lesions of twenty weeks duration the entire cartilage of the patellar groove was covered by pannus.

In all joints with dislocated patellae the articular surfaces were greatly altered. Marginal proliferation of articular cartilage had occurred (Fig. 4). Such proliferative changes were very marked in the joint which had been operated upon but four weeks previously (Fig. 5). The margins of cartilage in these joints were raised, scalloped and nodular. Numerous blood vessels and capillaries had grown inward from the adjoining synovial membrane. Histological examination revealed that the elevation of the articular cartilage margin was due in large part to the formation of new subchondral bone. In contrast to these proliferative changes there were areas of degeneration and atrophy of cartilage on the inner sides of the medial patellar ridges where the patellae had rested (Fig. 6). These proliferative and degenerative changes in articular cartilage, together with the overgrowth of subchondral bone, are similar to the changes encountered in human hypertrophic arthritis.

The dimensions of the surgically created defects in cartilage which were not obscured by pannus corresponded very accurately to the dimensions of the fragments of cartilage removed. Usually the margins of these defects were slightly rounded and less distinct in outline than when first made. In most instances they appeared slightly more shallow. All of the defects on the weight-bearing surfaces of the medial femoral condyles were more sharply defined than the majority of lesions in the patellar groove. This difference was due in part to the absence of any pannus overgrowth or fibrinous deposit in

the former location where pressure and friction of opposing articular surfaces may have retarded or prevented its formation.

The most recently made defect (four weeks) in the patellar groove was covered by a slightly adherent mass of fibrin. This fibrin clot was attached to the synovial membrane at the upper margin of the articular surface by two thin, narrow adhesions (Fig. 6).

The lesions in the patellar grooves of twelve and twenty weeks duration were completely or in part covered by a vascular connective tissue which could be seen in the gross examination to extend downward from the upper margin of the articular cartilage (Fig. 2).

The remaining joint in this experiment represented lesions of a twenty-eight week period. In this joint the patella was found to be in its normal position. Clinically there was no evidence of effusion and no excess of fluid was found when the joint was opened. There were no important changes from normal in the synovial membrane or articular cartilage. The defects in cartilage were very similar in gross appearance to the freshly made defects as viewed at operation (Fig. 7).

Microscopic study of the areas where articular cartilage had been worn away by the friction of the dislocated patella revealed flat even surfaces of uncovered subchondral bone which were polished and eburnated (Fig. 8).

Microscopic Examination of the Defects in Cartilage

Histological study of the lesions of four weeks duration on the weight-bearing and non-weight-bearing surfaces of cartilage revealed them to be very dissimilar. The lesion in the femoral condyle was an empty concavity extending down to the deepest one-third of cartilage. All of the cartilage cells had disappeared from a surrounding zone of matrix for a distance approximately equal to the width of the two columns of cartilage cells. In this acellular zone the cartilage matrix stained lightly and was fibrillated. Occasional recognizable lacunae from which cells had disappeared were seen. The surrounding columns of cartilage cells converged slightly toward the base of the defect. Several enlarged rounded clusters of cartilage cells were prominent in each section at the junction of the acellular zone of cartilage matrix and the deeper normal appearing cartilage (Fig. 9). The defect in the patellar groove was filled with a fibrillar mass

which stained in a manner characteristic of fibrin. The presence of such a fibrin clot seems readily explainable on the basis of injured capillaries and blood vessels where the subchondral bone had been exposed and traumatized by the abnormal position of the patella on the inner side of the medial patellar ridge (Fig. 6). This mass of fibrin was seen to be undergoing avascular organization by the ingrowth of fibroblasts at the margins of the defect. In addition to the sparsely disseminated fibroblasts, the fibrin clot contained a few scattered mononuclear phagocytes and occasional polymorphonuclear leucocytes. This defect was, in its greater part, surrounded by a lightly stained zone of cartilage matrix. Differing from the lesion already described, however, the margin of this defect near the surface of cartilage was not acellular (Fig. 10). In these regions oval and fusiform-shaped cells were present. They were often surrounded by lightly stained or unstained hyaline matrix. In a number of instances such cells appeared to have been entirely separated from surrounding intracellular substance and the impression was gained that some of them were extending by direct growth into the fibrin clot. In a few areas irregular depressions in the cartilage matrix at the superficial margins of the defects were filled with these fusiform cells; some were so intimately related to the cartilage matrix as to justify the conclusion that they arose from the original cartilage cells (Fig. 11). Serial sections through the two adhesions which extended from the synovial membrane above the articular cartilage into the upper portion of the fibrinous mass revealed that fusiform-shaped cells morphologically characteristic of fibroblasts were growing through these strands of fibrin. Thus it would appear that connective tissue cells were growing into the mass of fibrin from two sources, one the original articular cartilage, the other the synovial membrane at the upper margin of the articular surface. Such fibroblastic ingrowth appeared to be the first stage in one type of repair which was always encountered when subchondral bone had been injured or whenever sufficient intra-articular change had occurred as to result in pannus formation.

A number of blood vessels well filled with graphite extended into the deeper layers of the surrounding normal articular cartilage of this joint through gaps in the calcified zone. No other abnormalities were noted in the calcified cartilage, subchondral bone or marrow spaces.

In the joint representing lesions of twelve weeks duration the upper portion of the defect in the patellar groove was covered by vascular connective tissue "pannus" which extended onto the margin of the adjacent normal articular cartilage in a thin layer (Figs. 2 and 3). This pannus was slightly attached to the underlying cartilage by occasional fibroblastic-appearing cells which extended across the line of junction. In the lower one-half of the defect the gap in cartilage was filled with newly formed tissue which was intimately fused with the original cartilage and contained no injected blood vessels or capillaries. In places no separating line between original cartilage and recently formed tissue could be distinguished because of the close similarity of the newly formed intercellular material and the original cartilage matrix. Clusters and columns of well formed cartilage cells extended across the line which marked the boundary of the defect into the newly formed tissue. It was evident in this specimen that proliferation of cartilage cells had occurred (Fig. 12). The superficial cells of the repairing tissue had the morphology of fibroblasts and merged into the superficial cartilage at the margin of the defect. Examination of serial sections through the defect in the weight-bearing surface of this joint revealed that a few small blood vessels accompanied by fibrous tissue had grown in from the perichondrium at the articular margin. A number of these blood vessels had become thrombosed. The repairing tissue in its deepest layers resembled cartilage. In it were clusters of cells within lacunae. The matrix of the newly formed and old cartilage was fused. In several areas it appeared that the newly formed cartilage was growing out from old cartilage and that actual regeneration of cartilage had taken place. This impression was gained because clusters of cartilage cells extended across the line of fusion between the newly formed tissue and original cartilage. Some of these cells could not be distinguished histologically from those in the normal cartilage. There were no demonstrable alterations in the adjoining tissues.

The reparative processes in the lesions of twenty weeks duration were somewhat different from those already described. Vascular connective tissue filled the defect in the patellar groove. In this instance, however, it spread out in a thin layer over the entire articular surface. Although the tissue deepest in the defect slightly resembled cartilage, it was not intimately fused with original cartilage and there was no histological evidence that proliferation of cartilage cells

had occurred. The lesion on the weight-bearing surface of the femoral condyle was represented by a shallow depression, the deepest two-thirds of the defect having been filled with an avascular tissue which in its deepest portion was true hyaline cartilage (Fig. 13). Examination of a large number of sections through this lesion indicated more clearly than did the sections of previously described joints, that there had been proliferation of original cartilage cells (Fig. 13). Such evidence was present in the form of lengthened clusters of cells which extended across the line of fusion of the new and old matrix. These columns of cells converged toward the defect from their basal layers. Many of the individual cells had assumed elongated shapes and certain of these cells appeared immature in type.

In creating the lesions which were to represent reparative changes after twenty-eight weeks, the calcified zone of cartilage had been broken and the subchondral bone had been traumatized. In both of these lesions new bone trabeculae had been formed and proliferation of connective tissue into the defects from beneath had occurred. In the lesion on the medial condyle a small area of this repairing tissue resembled cartilage and a new layer of calcified cartilage was in process of formation (Fig. 14). In these lesions the margins of sectioned cartilage showed no evidence of repair by regeneration.

Experiment II. "Joint Mice" Composed of Hyaline Cartilage: Their Fate and Effect upon Intra-articular Tissues

Operation: The operative procedures used for these experiments were similar to those already described. A superficial strip of articular cartilage was removed from the patellar groove. Each fragment of cartilage was divided, one portion being returned to the joint as a loose body. The remaining portion was saved for histological examination.

Because of obvious displacement of the patella in the joint of the twelve week experiment, an additional joint was operated upon. A comparison of these two joints containing identical lesions of the same duration proved to be of interest. In the joint in which the patella had become displaced the knee joint was enlarged and contained about 5 cc. of viscid amber fluid. The patella was markedly atrophied and degenerated. There was a tremendous overgrowth of

synovial villi from the vascular portion of the synovial membrane. An exceedingly vascular pannus had overgrown the patellar groove. Numerous loose, white, rounded bodies ("joint mice") were floating free within the joint (Fig. 15). These loose bodies measured from 1 to 8 mm. in greatest diameter. It was evident that they had originated in the synovial membrane at its junction with the articular cartilage where many were in process of detachment. This observation was confirmed by histological examination. Microscopically the "joint mice" were seen to consist of circular, oval, or irregularly shaped masses of tissue which had an abundant amount of hyaline intercellular material. In some portions this intercellular material showed a fibrillar background. Several layers of cells, having the morphology of fibroblasts, paralleled the surface of these bodies. In the central portion of some of them, grouping of cells into pairs and clusters had occurred so that by virtue of their morphology and arrangement they resembled cartilage cells (Fig. 16). Very few mitotic figures were found in the peripheral layers of fibroblasts, indicating that these bodies were growing with considerable rapidity although floating free within the joint space. Thrombosed blood vessels were present within occasional floating bodies, indicating as did the gross appearances, that they had taken origin in the hypertrophied villi at the articular margins. One loose body which was thin and oval in shape was histologically consistent with the original implanted fragment of cartilage, although considerable alteration in its structure had taken place. The cartilage cells were less evenly placed in the matrix than normal, a number of them were elongated, and at the periphery at one end of the fragment there was evident proliferation of fusiform cells. This fragment was entirely avascular.

All of the remaining knee joints used in this experiment, including the one which was substituted for the original twelve week experiment, were relatively normal. The patellae were normal in relation to the other structures. None of the joints contained a demonstrable excess of fluid and there was no important intra-articular pathology other than a slight hypertrophy of the synovial villi. There was no appreciable macroscopic evidence of healing of the defects in cartilage in any of these joints.

The fragments of cartilage which were placed within the joints were recovered in three specimens and verified by histological examination. One of these fragments was free within the joint. Al-

though the cartilage cells were viable, there had been considerable alteration in their arrangement, and growth of cells which had the appearance of fibroblasts was seen at one end of the fragment. The other cartilage implants had been in large part surrounded by the synovial membrane of the fat pad below the patella. One of these fragments stained in a manner characteristic of viable cartilage and the majority of the cartilage cells appeared normal. There were, however, a considerable number of empty lacunae from which cartilage cells had disappeared (Fig. 17). The remaining fragment of cartilage which was identified was of the same shape and size as when implanted. In it a large number of cartilage cells had degenerated, others were shrunken and contained pyknotic nuclei and the matrix was faintly stained. None of these cartilage "mice" had become vascularized.

Histological examination of the defect in cartilage of four weeks duration showed neither evidence of cartilage regeneration nor any other type of repair. The defect had remained as an empty concavity surrounded by a lightly stained zone of matrix from which the cartilage calls had disappeared. Death of cartilage cells had likewise occurred in the superficial portion of the articular cartilage of the patellar groove at the edges of the defect. This defect extended down to but did not include any of the calcified zone of cartilage. The remainder of the femoral articular surface appeared normal in all respects.

The defect in cartilage from the joint of the twelve week experiment was almost identical in its microscopic appearance to the one already described. In this joint, however, the cartilage cells appeared entirely normal in every portion of the joint except at the immediate margin of the defect crater. A slight amount of fibrosis of the marrow tissue immediately below the lesion in cartilage had occurred.

In the joint representing the twenty week experiment the defect extended through the calcified zone of cartilage into the superficial subchondral bone. The base of the defect was covered by avascular fibrous tissue which was about twice the thickness of the calcified zone of cartilage. This fibrous tissue resembled cartilage in its deepest layers. New bone trabeculae had been built up beneath the defect and it was evident that the fibrocartilage was still being replaced by bone. A zone of calcified cartilage had begun to reform. The

margins of the defect were sharply outlined. A narrow zone of unstained cartilage matrix formed an easily recognized boundary where original cartilage had been removed. For the most part this zone of matrix was acellular; however, a few clusters of cells from the original cartilage were extending through it to lose their identity in the newly formed repairing tissue. There was no evidence of cellular proliferation at the margins of the defects in the superficial one-half of the articular surface where there was no adjoining newly formed fibrous tissue.

The defect from the joint operated upon twenty-eight weeks previous to examination showed slight reparative changes. The defect was in large part lined by unstained matrix; however, in the depth of the crater, cartilage cells had grown into this zone from beneath to form clusters of from six to twelve cells each (Fig. 18). Several of these groups of cells were enclosed in thin-walled lacunae (Fig. 19), while in other fields the surrounding deeply stained margin of the matrix had disappeared and the cells were extending into the newly formed tissue which covered the base of the defect (Figs. 20 and 21). The base of this defect, which was entirely within articular cartilage, was covered by recently formed repairing tissue about the thickness of the calcified zone of cartilage. This repairing tissue was, on the surface, morphologically characteristic of fibrous tissue (Fig. 19). However, in the deepest portion it had the histological appearances of cartilage (Fig. 20).

Experiment III. "Joint Mice" Composed of Hyaline Cartilage and Subchondral Bone

The operative procedure for this experiment was identical with that used in the preceding one except that subchondral bone was removed to an approximate depth of 2 mm. with the overlying cartilage. The fragments were divided and one-half of each fragment was returned to the joint. Care was taken to prevent the escape of blood into the joint space by delaying closure until all oozing from the subchondral blood vessels had been controlled.

Pathological Examination: The joint representing the lesion of four weeks duration showed no prominent intra-articular changes aside from the unhealed defect in the patellar groove. However, a swelling within the joint capsule was noted. This swelling contained

a cavity which communicated with the joint space by a small sinus tract. Within the cavity were several small fragments of tissue which appeared to be the remains of the implanted loose body. It was apparent that the false opening from the joint space was due to imperfect healing of the surgical incision.

In the joints containing lesions of twelve and twenty weeks duration, as in the preceding joint, there were no important intra-articular changes from normal accompanying the defects and "joint mice" (Fig. 22). In all of these joints the patellae were found to be in their normal positions. The defects in the patellar grooves were sharply outlined (Fig. 22).

It should be emphasized again that in preceding experiments, where no patellar displacement had occurred, no important intra-articular pathology was found (Fig. 23).

In contrast to the above joints the patella in the twenty-eight week specimen was displaced so as to overlie the inner patellar ridge. As in similar instances already described, this abnormality was accompanied by extensive proliferative changes in the synovial membrane (Fig. 24), and both proliferative and degenerative changes in the cartilage of the femoral and patellar surfaces. The defect in the joint was obscured by a mass of vascular connective tissue. The fragment of cartilage and bone which had been implanted in this joint was not found.

In the joint representing the lesion of cartilage and bone after twelve weeks, the implanted fragment was found unattached in a small concavity of the fat pad just below the patella. There had been considerable absorption of bone, all of the bone cells had degenerated, and the marrow spaces were filled with fibrous tissue which resembled cartilage. There was no evidence of any bone formation, neither was there evidence of proliferation of endosteal cells. The hyaline cartilage of the fragment had remained viable in its entirety. It showed no regressive changes in either cells or matrix. This entire loose body was surrounded by a narrow layer of proliferating fibroblasts.

The loose body in the joint operated upon twenty weeks previously was partially surrounded by synovial membrane. The bony portion of this fragment had been absorbed, whereas the cartilage matrix was well preserved. The cartilage cells in the greater part of the fragment had maintained a normal appearance.

Microscopic study of the defects in these joints revealed that bone had been removed by surgical means to a depth of about twice the thickness of articular cartilage. In the earliest lesion, that of four weeks duration, the defect was represented by a deep, sharply outlined depression. At the defect margins there was evidence of great osteoclastic resorption of injured bone trabeculae with practically no evidence of bone formation. The surrounding marrow spaces were filled with vascular connective tissue which was continuous with immature connective tissue that filled the deepest portion of the defect. The margins of sectioned articular cartilage were sharply defined, acellular, and there was no histological evidence of proliferation of cartilage cells (Fig. 25). The defect of twelve weeks duration was more shallow. There was less osteoclastic resorption of original bone to be seen. New bone formation was present, as was shown by the thickening of the adjoining original bone trabeculae and the presence of numerous osteoblasts. The newly formed bone merged into the fibrous tissue which in the deepest portion of the defect resembled fibrocartilage more than in the preceding joint. The surrounding marrow spaces were filled with a very vascular fibrous tissue. In this specimen the fibrous tissue which filled the defect was fused with the deepest layer of articular cartilage at the defect margins (Fig. 25). There was, however, no evidence of proliferation of cartilage cells and the margins of sectioned cartilage were covered by light staining hyaline matrix from which the cells had disappeared.

The older defects, twenty and twenty-eight weeks duration, in this experiment had become more shallow since the greater portion of the concavity had been filled in with bone. Such bone was composed of thick, irregularly placed trabeculae. The intertrabecular spaces were largely filled with fibrous tissue and a considerable amount of apposition of bone by osteoblastic activity was present. The superficial layer of recently formed bone merged imperceptibly into the dense connective tissue and fibrocartilage which formed the surface tissue in the defect. This fibrocartilage was fused intimately with the articular cartilage at the defect margin (Fig. 26). The only histological difference between the repairing tissue and the original cartilage was that in the recently formed tissue the cells lacked characteristic grouping into lacunar spaces and columns. After studying the above sequence of changes one is forced to conclude that this newly formed and imperfect cartilage developed through stages of

metaplasia from the typical fibrous tissue which originally filled the defect (Fig. 25). Such fibrous tissue probably took origin in the connective tissue of the marrow spaces in the subchondral bone.

Experiment IV. Changes in Articular Cartilage Associated with the Removal of Opposing Articular Surfaces

After unsuccessful attempts had been made to maintain separation of articular cartilage surfaces within unopened joints, disarticulation with careful closure of the synovial membrane and joint capsule over the exposed femoral articular surfaces was resorted to in an attempt to obtain some information as to the importance of apposition of cartilage surfaces in maintaining normal nutrition. The patellae and patellar ligaments were utilized in covering the denuded articular ends. The approximation of the margins of the flaps to reform a synovial-lined space proved fairly satisfactory. Specimens of twelve and twenty-eight weeks duration were obtained for study by this method. There was no material difference either in type or degree of the changes from normal which occurred in these specimens.

Pathological Examination: When the synovial membrane was incised at the margins of the articular cartilage, fine "cobweb" and coarse adhesions were encountered. These adhesions were present in several areas although there were numerous small synovial spaces remaining. The uncovered surfaces of articular cartilage were nonglistening, gray in color and showed numerous areas of partial atrophy or complete degeneration.

When gross sections of cartilage and subchondral bone were made it was noted that the cartilage was very thin, even in the least changed areas, and that there was marked atrophy of the subchondral bone.

Microscopic examination revealed that very marked thinning and decalcification of the subchondral bone trabeculae had occurred. The articular cartilage was everywhere thinned out. In places it had completely degenerated. In a number of areas the calcified zone of cartilage was much thinner than normal. In some of the sections the surface of articular cartilage was covered by a thin layer (five to ten cells deep) of fibroblasts which could be traced to the perichondrium

at the margins of the cartilage. All of the sections showed superficial cartilage depressions and areas in which the cartilage cells had degenerated, leaving lightly stained and slightly fibrillated cartilage matrix. In the midzone of articular cartilage many of the cartilage cells had become fusiform in shape and occurred singly or in clusters. This finding serves to emphasize the fact that mature cartilage cells may acquire, under appropriate stimulus, the morphology of fibroblasts.

Discussion

Although numerous workers have studied the repair of defects made in hyaline cartilage of articular surfaces, there has been no substantial agreement of opinion regarding the ability of cartilage to regenerate, or by what method repair of cartilage occurs. It is probable that the existing confusion is due to the facts that in most publications no clear differentiation has been made between repair by proliferation of connective tissue from neighboring tissues and independent regeneration of cartilage; that the repair of lesions of cartilage with injury to subchondral bone have not been separated from the repair of lesions made entirely within articular cartilage; and that much of the work from which deductions have been drawn was done before the time when the importance of strict asepsis in joint operations was realized.

Since an inclusive review of the literature pertinent to the regeneration of hyaline cartilage has been recently recorded by Shands,[2] references to previous work will be minimized in this report. One may divide the opinions of earlier authors into three groups: (1) those who believe that independent regeneration of adult articular cartilage does occur; (2) those who insist that cartilage does not have the ability to regenerate, and (3) those who believe that regeneration occurs through proliferation of fibroblasts with subsequent metaplasia into cartilage.

1. Seggel[3] reported that within twenty-four and forty-eight hours after a defect in cartilage had been made, the cartilage cells directly adjacent to the defect became swollen, that small cartilage islands were formed mostly in the center of the defect and that after twelve days mitoses were found. He noted that infection checked this reaction and that it was less marked in older animals. It was also observed by this author that defects near ligament or membrane attachments became covered by pannus and that centrally

located shallow defects and linear incisions showed no reparative reaction after long periods of time. Fasoli [4] described degenerative changes in surrounding cartilage cells and matrix immediately following injury. At a later time he noted proliferative changes in cartilage cells at the margins of the defects with division of cartilage cells by mitosis after nine days. He described slow, progressive, proliferative changes until complete repair had occurred. Even after six months time he found doubtful evidences of continued regeneration around the defect margins.

2. Haebler [5] concluded from his experiments that defects in articular cartilage which did not include subchondral bone showed no evidence of healing from the borders of the defects within 304 days. He further concluded that when connective tissue or fibrocartilage was found filling the injured cartilage, serial sections would reveal that subchondral bone had been injured in some small area. It was also the opinion of Geis [6] that clean aseptic wounds in cartilage do not heal and that cartilage does not possess the power of regeneration. Geis, however, did find that in the presence of infection healing of cartilage occurred so as to leave little or no evidence of the defect. The ability of hyaline cartilage to regenerate in adult mammals is denied by Maximow and Bloom.[7] They state that wounds repair by the ingrowth of connective tissue from the perichondrium or the nearest fascia and that the failure of independent regeneration of cartilage is due to the inability of mature mammalian cartilage cells to divide mitotically. In tangentially placed superficial wounds of cartilage which did not extend into subchondral bone, Ciociola [8] observed scarcely any reaction. He did observe the repair of wounds in cartilage which extended into subchondral bone. Such repair occurred by connective tissue proliferation and transformation into hyaline cartilage.

3. Healing of wounds in articular cartilage, by the proliferation of fibrous tissue from one of a number of sources, has been described by several authors. Redfern [9] in 1851 stated that wounds in articular cartilage heal perfectly by fibrous tissue, which he believed to arise from the intercellular substance and cells of the articular cartilage. Gurlt [10] concluded that defects in cartilage are repaired by a fibrous, and at times cartilage-like tissue, but that it is never completely replaced by cartilage and true regeneration of cartilage does not occur. Fisher [11] reported that greater regenerative ability of

cartilage existed at the margins of the articular surfaces as compared to the central areas. He explained this difference on the basis of better nutrition and the presence of perichondrium in the former location. However, no clear distinction between the repair of those lesions in which subchondral bone had been injured and those in which only cartilage had been traumatized was made. More recently Shands,[2] after studying the repair of lesions in articular cartilage in dogs, came to the conclusion that cartilage did not regenerate in less than four weeks and that when regeneration did occur it progressed through stages of fibrin formation, granulation tissue, fibrous tissue and transformation of fibrous tissue into cartilage. He was unable to demonstrate any difference in the regenerative powers of cartilage in the various areas of the articular surfaces. Key [12] and Ito [13] observed that repair of defects in hyaline cartilage and subchondral bone occurred by the proliferation of fibrous tissue from the marrow spaces, with subsequent transformation of the fibrous tissue into cartilage. Attention was called to the observation that the injured surfaces of both bone and cartilage die and that repair occurs through the proliferation of the osteogenic cells lining the marrow spaces.[12]

The present series of experiments indicate that adult articular cartilage does have a limited ability to repair aseptic lesions within its substance by independent regeneration of cartilage. The powers of such regeneration, however, are feeble and not always demonstrable. The greatest regenerative activity was noted in defects on the weight-bearing surfaces of the femoral condyles, whereas a lesser proliferative activity was found in the non-weight-bearing surface of the patellar groove. It was in the latter location that no reparative reaction was seen in two joints where the lesions had been present for periods of four and twelve weeks. A satisfactory explanation of this complete absence of regeneration in the two lesions described is not possible. The fact that none did occur, however, serves to emphasize the feeble ability of cartilage cells to proliferate, particularly the cartilage cells which are most distant from the perichondrium at the articular margins. It is only fair to point out the possibility that the animals in which no repair occurred may have been older than the others, since the exact ages of the dogs could not be ascertained. Only adult dogs * showing no evidences of advanced age were se-

* The repair of articular cartilage in young dogs before epiphyseal union has occurred will be commented upon in a subsequent report.

lected for use. When proliferative changes do occur in defects in cartilage, one finds a convergence of cell columns toward the base of the defect with the formation of superficial clusters of cartilage cells to indicate that original cartilage cells have multiplied within lacunae. No indication as to the manner of division of these cells was obtained from these experiments, although it should be noted that mitotic figures were observed by other workers after nine [4] and twelve days.[3] No lesion in the present series was examined before a duration of four weeks. The sequence of changes which appeared to have occurred were the projection of such clusters of cells into the acellular zone of cartilage matrix at the margin of the defect, with disruption of the lacunar margins and the spread of cartilage cells over the surface of the defect. In several instances a continuation of cartilage cell columns across the line of junction of the new and original tissue could be traced. Ultimately the newly formed tissue within the defect developed, by virtue of the amount and staining quality of its intercellular substance and the morphology of its cells, a distinct resemblance to hyaline cartilage. Perfect hyaline cartilage, however, was not reformed in these lesions.

A different form of repair occurred in the defects of the patellar groove in the joints where the patellae had become displaced. In these instances vascular connective tissue (pannus) spread over the defects from the articular margins. The earliest changes of this sort were observed in a lesion of four weeks duration. The defect was filled with a mass of fibrin into which fibroblasts were growing from the superficial levels of articular cartilage at the margins of the defect and from the synovial membrane at the upper margin of the articular surface. A careful histological study of the articular cartilage at the immediate margin of the defect revealed that lightly stained or unstained cartilage matrix surrounded scattered cartilage cells. A number of these cells in each section appeared to have been liberated entirely from hyaline matrix and the impression was gained that some of them were growing into the fibrin clot and were therefore in part responsible for the early avascular organization which was occurring. Cartilage cells have been grown in tissue culture [14] and it is not illogical to assume that a similar type of growth may occur within joints. The great variety of ways in which mesenchymal cells may differentiate or dedifferentiate because of location and function, causes one to consider also the possibility of detached

synovial cells becoming implanted in such a mass of fibrin, growing as fibroblasts and thus taking part in the organizing process. The fibrin which formed within this and other joints probably resulted from injury to capillaries and blood vessels where cartilage and subchondral bone were worn away by the displaced patellae. Through a continued connective tissue growth and proliferation of vascular endothelium from the articular margins, pannus was eventually formed. The defects, and later the surface of cartilage, became covered by vascular connective tissue in the non-weight-bearing surfaces of the joint. In the defects, the deepest layer of pannus later became transformed into tissue that histologically resembled fibrocartilage. In one lesion in the patellar groove independent repair by proliferation of cartilage cells was apparent in the lower one-half of the defect, whereas in the upper one-half the repairing tissue was being absorbed in the pannus which was growing downward from the synovial membrane at the upper margin of the articular surface.

A third type of repair took place in those lesions which extended into subchondral bone. In such instances a proliferation of connective tissue from the marrow spaces occurred. Proliferating fibroblasts, accompanied by fairly numerous blood vessels, filled the deepest portion of the concavity of the defect and the surrounding marrow spaces. In the older lesions it was noted that a great deal of intercellular substance had been formed by the fibroblasts. In the deepest portions of the defects the newly formed tissue had been transformed into bone. The new bone merged into an intermediate layer of dense, rather avascular connective tissue, while on the surface the repairing tissue had acquired the morphological appearance of imperfect hyaline cartilage. The original bone trabeculae about the margins of the defects had been widened by osteoblastic activity and the marrow spaces had maintained a richer blood supply than normal. In occasional specimens a new zone of calcified cartilage had partially reformed and extended into the repairing tissue from the calcified zone of cartilage at the margin of the defect. It was not until periods of twenty or twenty-eight weeks had elapsed that the defect crater had in large part been filled. At that stage the surface layer of newly formed tissue was avascular, resembling cartilage in the amount and staining quality of its intercellular substance and in the morphology of its cells. Although the matrix of the new cartilage was fused with the matrix of the original cartilage, the new cells

did not acquire the usual distribution in columns such as is characteristic of normal articular cartilage.

The fragments of articular cartilage "joint mice" which had been returned to the joints from which they were removed were found to contain a few empty lacunae from which cells had disappeared. The majority of cells, however, were viable and the greater part of the matrix stained with normal intensity. The entire fragments were found to be of essentially the same shape as when implanted. In most instances they had been surrounded by vascular connective tissue of the synovial membrane and thus removed from the joint space. The tendency for removal of fragments of cartilage from the articular space was noted by Ito [13] in experimenting with rats. He believed that the small size of the joints in his experiments may have been an important factor in causing their removal. In the present experiments, the bone of the cartilage and bone implants had been destroyed or was in process of being removed. In these same fragments the cartilage had not undergone necrosis, and in one instance showed no evidence of any retrograde change. It is a noteworthy fact that this fragment had remained entirely free within the joint for a period of twelve weeks, in contrast to the less well preserved fragments of cartilage which had been removed from the joints. Harbin and Moritz [15] described the survival of collodion-encased fragments of articular cartilage in knee joints for as long as thirty-two days.

The findings from the present series of experiments indicate that neither the presence of surgically created defects within cartilage or cartilage and bone, nor the presence of loose bodies of cartilage or cartilage with attached bone are, in themselves, a cause of important associated intra-articular pathology. Such an observation is not in agreement with the conclusions of Key [12] who reported inconstant, but at times marked joint changes of the hypertrophic type in rabbits from which he had removed small pieces of cartilage and underlying bone. He was unable to explain the variability of the pathological changes which followed these similar operations, although he was of the belief that they were due to the presence of the surgically made defects. Haebler [5] associated arthritic changes in experimental joints with displaced patellae. In the present group of experiments important intra-articular changes of a type similar to the changes in hypertrophic arthritis of man were encountered in every joint in

which the patella had become permanently displaced following the displacement at operation. Such changes did not occur in any of the joints in this series where the patella remained in its normal position. The occurrence of spontaneous and progressive defects in articular cartilage and subchondral bone in bovine joints [16] without other important intra-articular changes is further evidence that the defects themselves are not a cause of other important joint changes. Associated with the patellar dislocation, each joint of the present series showed marked hypertrophy of the synovial villi and marked marginal "lipping" of cartilage due to proliferation of the subchondral bone. Atrophy and degeneration of cartilage occurred where the patella had moved about in its abnormal location, and polishing with eburnation of the uncovered bone followed the degeneration of cartilage. In each of these joints, pannus, which was made prominent by the intravascular injection of graphite ink, developed on the non-weight-bearing surfaces of cartilage. It is a noteworthy fact that pannus did not extend over the weight-bearing surfaces of the condyles in the joints in which it had formed in other areas. This fact indicates that weight-bearing and motion of the opposing articular surfaces may retard or prevent the formation of pannus. "Joint mice" developing from hypertrophied and detached synovial villi occurred in most of these greatly altered joints.

The atrophy and degeneration of cartilage with pannus overgrowth that occurred in joints in which the femoral articular surfaces were removed from contact with other cartilage by amputation through the knee joint was similar to that observed by others.[5, 11] These findings suggest that the apposition of one articular surface with another is probably important in the maintenance of normal hyaline cartilage.

Summary

1. Studies concerning the repair of surgical defects made in hyaline cartilage of normal adult dog joints, the joint reaction to loose bodies of cartilage and cartilage with attached bone, and the joint reaction to displaced patellae are reported. Each type of lesion was examined after periods of four, twelve, twenty and twenty-eight weeks duration.

2. Some form of repair occurred in seven of the nine defects which were made entirely within the articular cartilage of the weight-bear-

ing and non-weight-bearing articular surfaces. The two exceptions were represented by lesions in the patellar groove of four and twelve weeks duration.

3. In the defects which extended into subchondral bone and in those defects where pannus, accompanying displaced patellae, covered the defects the reparative changes passed through stages of fibrous tissue and fibrocartilage to the formation of an imperfect form of hyaline cartilage. The fibrous tissue originated in the connective tissue of the bone marrow, in the marginal synovial membrane and apparently, in some instances, from articular cartilage cells.

4. Histological evidence of repair of cartilage by proliferation of cartilage cells was present in four of the six defects which were entirely within cartilage and not covered by pannus. Such proliferation was most marked in the lesions made in the weight-bearing surface of the femoral condyle. In no instance was repair complete or perfect within the twenty-eight week period. In the majority of lesions the repairing tissue filled but a small portion of the defect crater.

5. In these experiments marked intra-articular changes similar to those of human hypertrophic arthritis occurred in every joint in which the patella became displaced. Such joints showed pannus formation, hypertrophied synovial villi, "joint mice" formation and proliferative and degenerative changes in the articular cartilage and subchondral bone at the articular margins.

6. The presence of small defects in cartilage, or defects which extended into subchondral bone, was not a cause of important joint pathology in these experiments.

7. Cartilage and bone and cartilage fragments returned to the joints from which they were removed did not produce any significant intra-articular changes. The bone of the bone and cartilage fragments had been resorbed or was in the process of resorption, whereas the cartilage had remained viable in large measure in all instances. In the majority of specimens the implanted loose body had been surrounded by connective tissue and thus removed from the intra-articular space.

8. Extensive atrophy of cartilage and pannus formation over the surface of cartilage occurred within a twelve weeks period, when disarticulation through the knee joint was performed. These findings

suggest the importance of the apposition and weight-bearing of adjoining articular surfaces in maintaining the proper nutrition of hyaline cartilage.

9. The application of a method of capillary and blood vessel injection with a substance, which is easily recognized on macroscopic and microscopic examination, is described.

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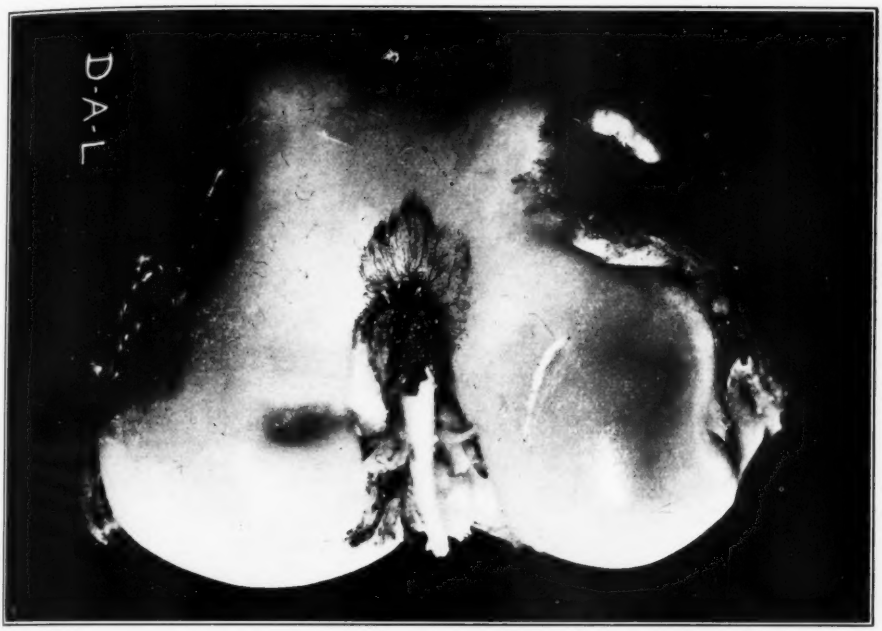
DESCRIPTION OF PLATES

PLATE 88

FIG. 1. A gross photograph of the articular surface of the femur, showing pannus at the synovial margin of the intercondyloid notch and at the lateral margin of the patellar surface. The blood vessels are filled with graphite ink. The surgically made defect of four weeks duration is visible on the medial condyle. × 2.5.

FIG. 2. An anterior view of the articular end of the femur showing surgical defects of twelve weeks duration. Associated with displacement of the patella the articular surface has become widened and elevated at the margins. Note the extension of pannus from the upper margin of cartilage on to the upper one-half of the defect in the patellar groove. × 2.5.



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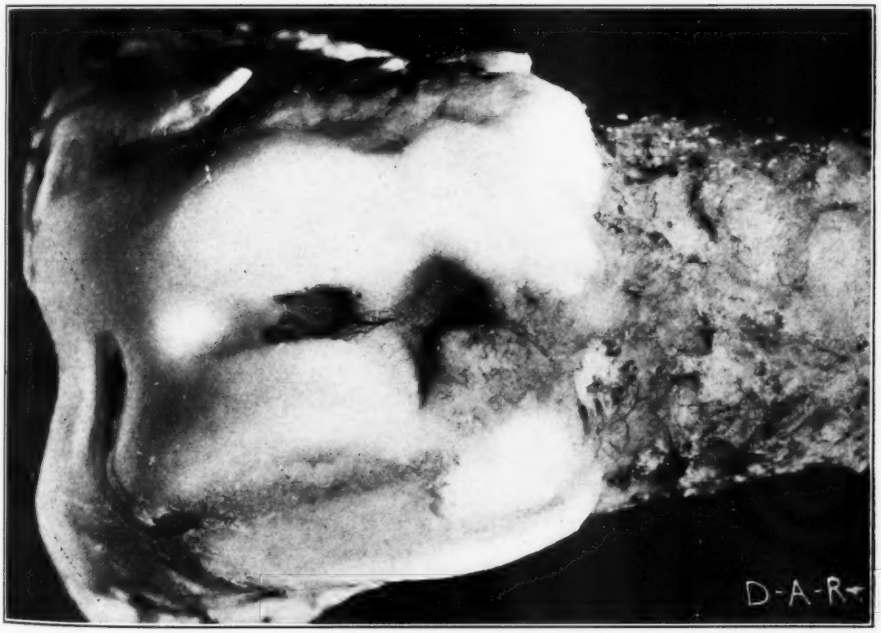

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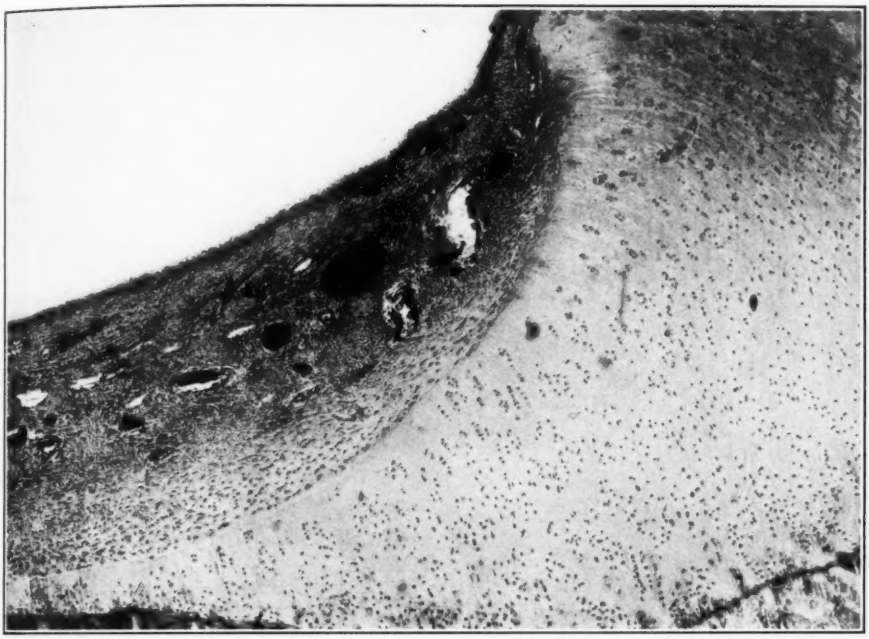
PLATE 89

FIG. 3. A photomicrograph which shows the changes in one-half of the twelve weeks old defect in the patellar groove. Note the numerous graphite-filled blood vessels in the pannus, the formation of fibrocartilage in the deepest layer of repairing tissue and the sharply outlined and acellular margin of the defect. The section was made through the upper one-half of the lesion into which blood vessels had extended (Fig. 2). × 76.5.

FIG. 4. Proliferative changes in articular cartilage of the hypertrophic type are illustrated in this photograph of a joint which contained defects of twelve weeks duration. The patella was displaced during the entire twelve week period. × 2.5.



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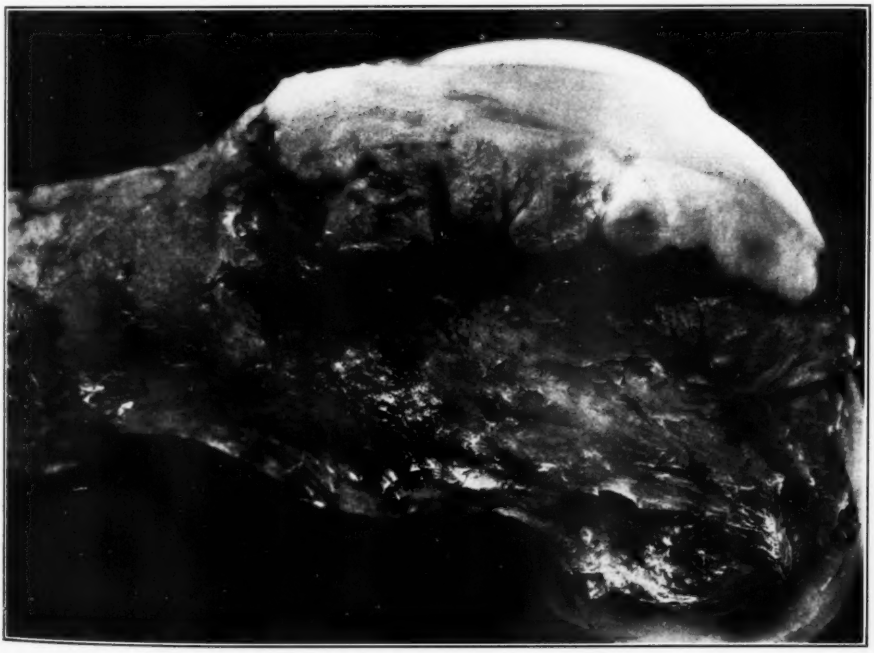


4

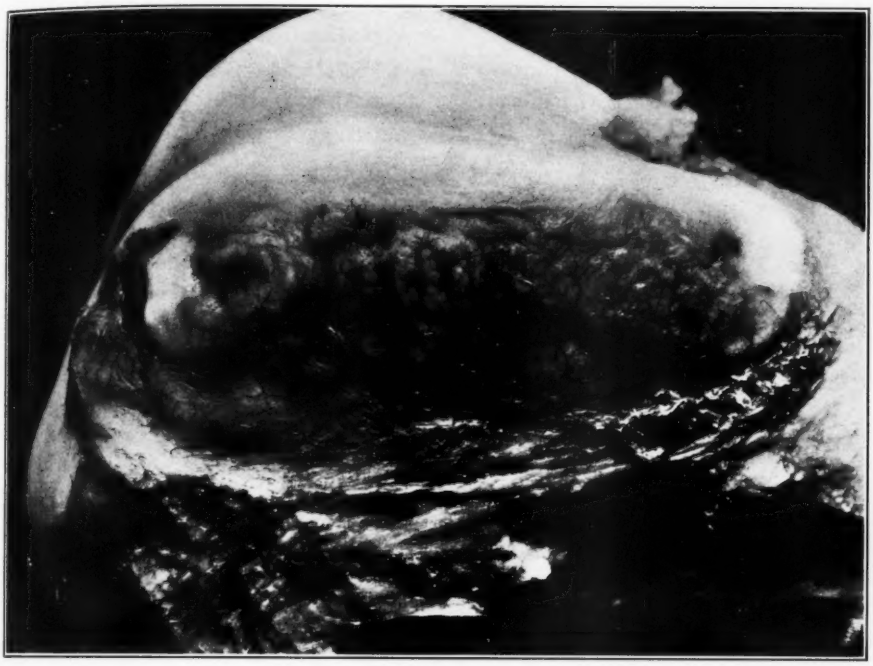
Bennett, Bauer and Maddock Repair of Articular Cartilage

PLATE 90

FIG. 5. A lateral view of the femoral end of a joint operated upon four weeks earlier. The patella became displaced and, as was invariably the rule, proliferative and degenerative changes in articular cartilage occurred. Note marginal proliferation, elevation and vascularization of cartilage. × 2.5.

FIG. 6. A photograph of natural size, showing atrophy and degeneration of articular cartilage on the medial side of the joint where the patella had rested. The defect in the patellar groove is covered over by an avascular and partially organized fibrin clot which is attached to the synovial membrane at the upper margin of the articular surface by two adhesions.

FIG. 7. A photograph of natural size, showing the sharply outlined surgical defects in the cartilage of the patellar groove and femoral condyle after twenty-eight weeks duration. The patella was not displaced and the joint remained essentially normal.



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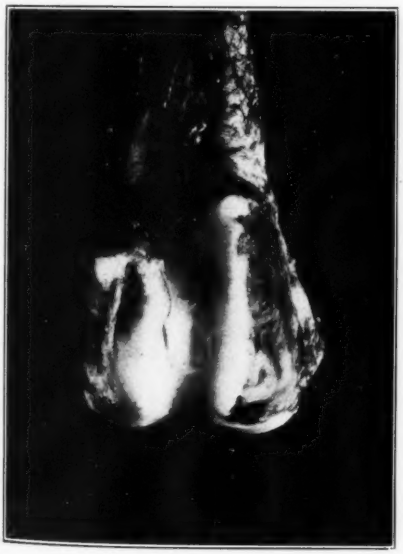

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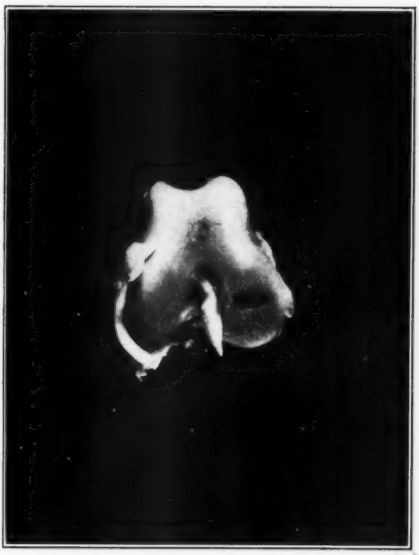

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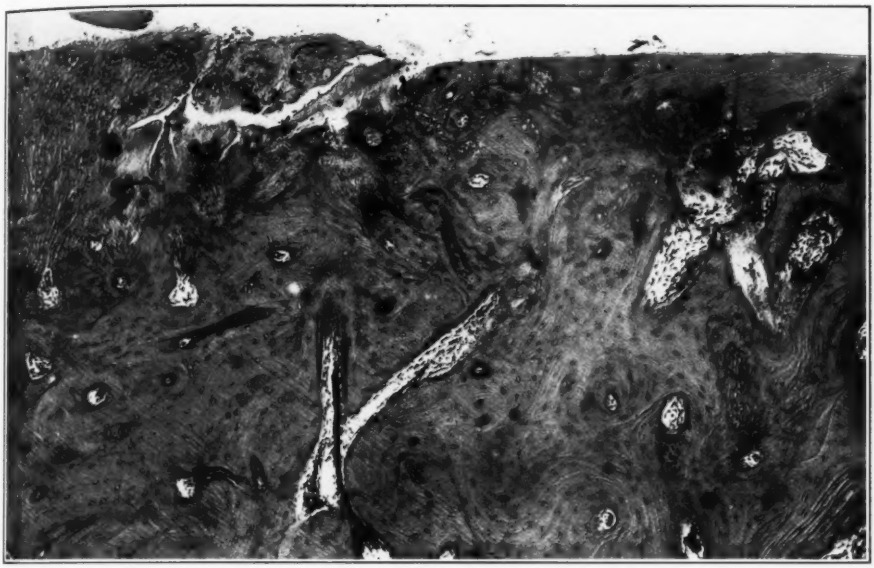
PLATE 91

FIG. 8. A photomicrograph showing eburnation and polishing of bone where the articular cartilage has been worn away during a four weeks period by the dislocated patella. × 76.5.

FIG. 9. A section through the entire defect in the cartilage of the weight-bearing condyle is shown in this photomicrograph. The lesion was present for four weeks. Note the acellular margin of the defect in the deepest portion, the converging columns of cartilage cells and the formation of new cartilage in the superficial one-half of the defect crater. × 76.5.



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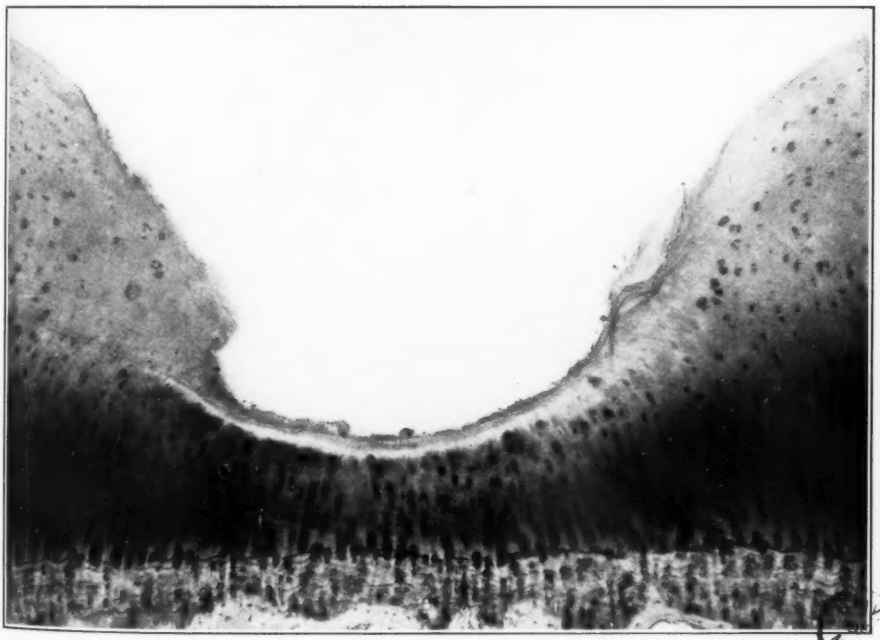

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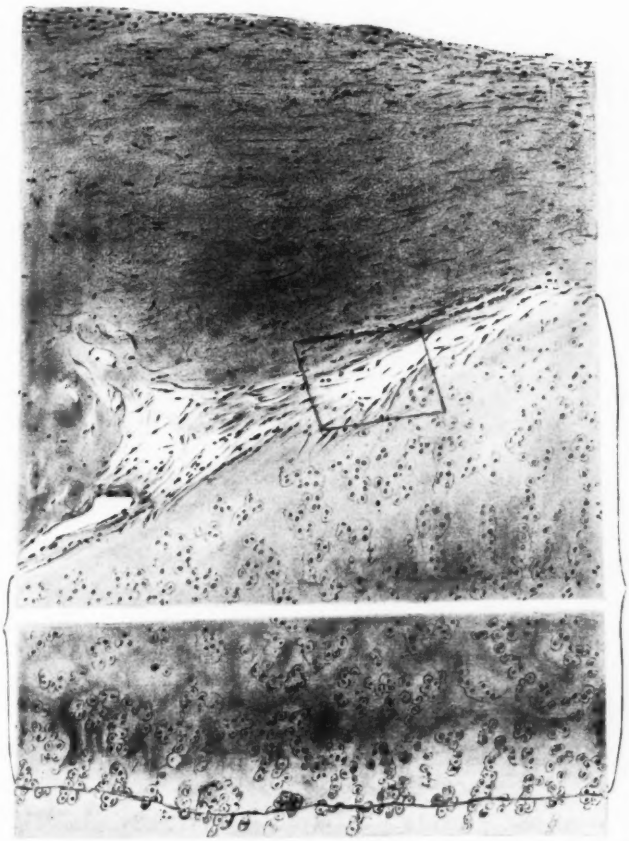
PLATE 92

FIG. 10. A camera lucida drawing showing the margin of a defect of four weeks duration in the patellar groove. The defect is filled with fibrin undergoing organization. Note apparent proliferation of fibroblasts from the cells in the superficial layers of the original articular cartilage. × 170.

FIG. 11. A camera lucida drawing of the inset in Fig. 10. × 420.



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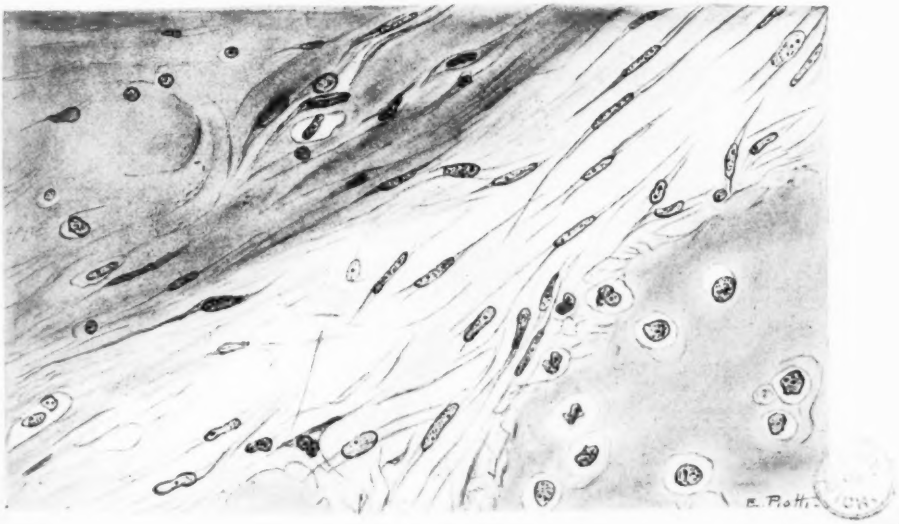

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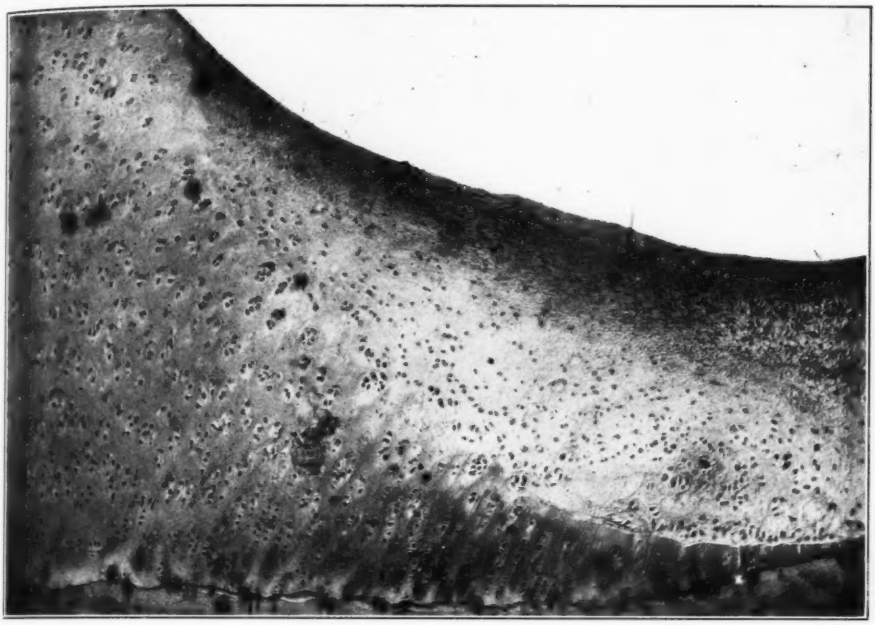
PLATE 93

FIG. 12. One-half of the defect of twelve weeks duration in the cartilage of the patellar groove is shown in this photomicrograph. Note extension of clusters of cartilage cells across the boundary between the original cartilage and recently formed tissue filling the defect. Near the surface the repairing tissue appears to be fibrous tissue; however, in the deeper layers, it resembles hyaline cartilage. × 68.5.

FIG. 13. Proliferation of cartilage cells into the defect crater is shown clearly in this photomicrograph of a section from a twenty weeks old defect in the cartilage of the femoral condyle. × 76.5.



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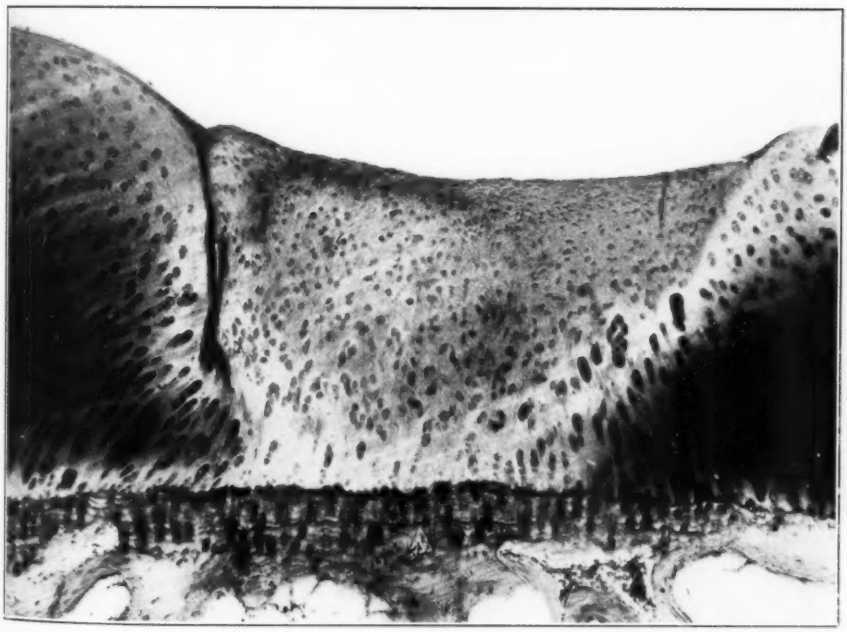

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PLATE 94

FIG. 14. A photomicrograph showing the repair in a defect of twenty-eight weeks duration which extended into subchondral bone. The defect is filled with fibrous tissue, fibrocartilage and imperfect hyaline cartilage. A new zone of calcified cartilage has partially reformed. Note the acellularity of the matrix of original cartilage at the margin of the defect. × 76.5.

FIG. 15. A gross photograph of natural size showing panus covering the surface of the patellar groove and the "joint mice" which formed within the joint. Note the widened and uneven articular surface. These changes which accompanied dislocation of the patella occurred within a period of twelve weeks.

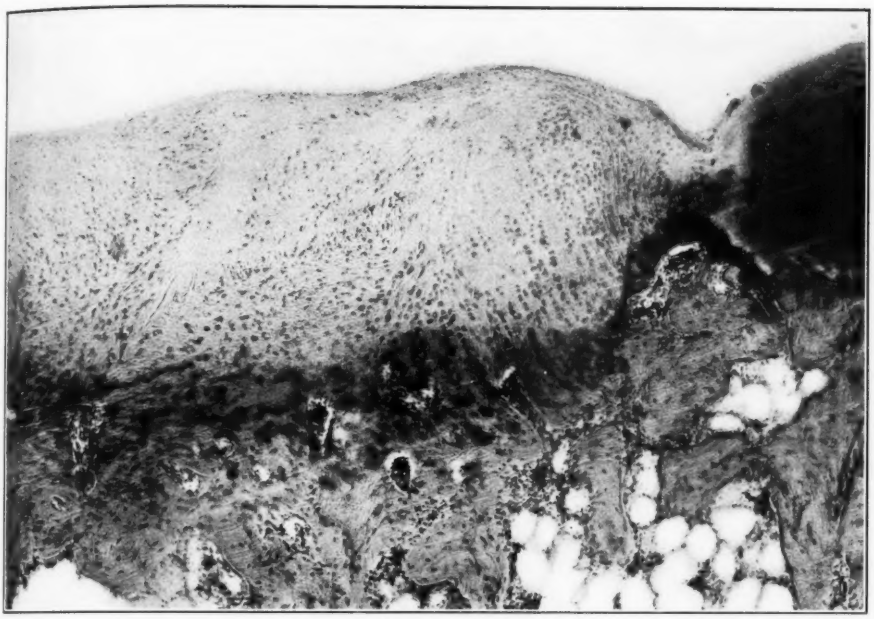

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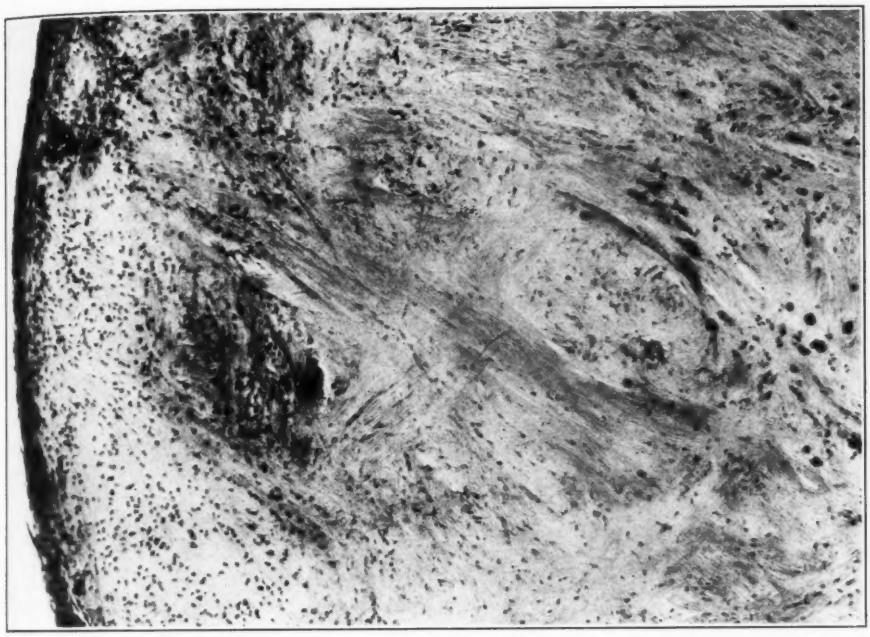
PLATE 95

FIG. 16. A photomicrograph showing the structure of the largest "joint mouse" illustrated in Fig. 15. × 76.5.

FIG. 17. A photomicrograph which illustrates how implanted fragments of articular cartilage often were surrounded by the vascular connective tissue of the synovial membrane. The fragment of cartilage is in large part viable after a period of twelve weeks within the joint. × 76.5.



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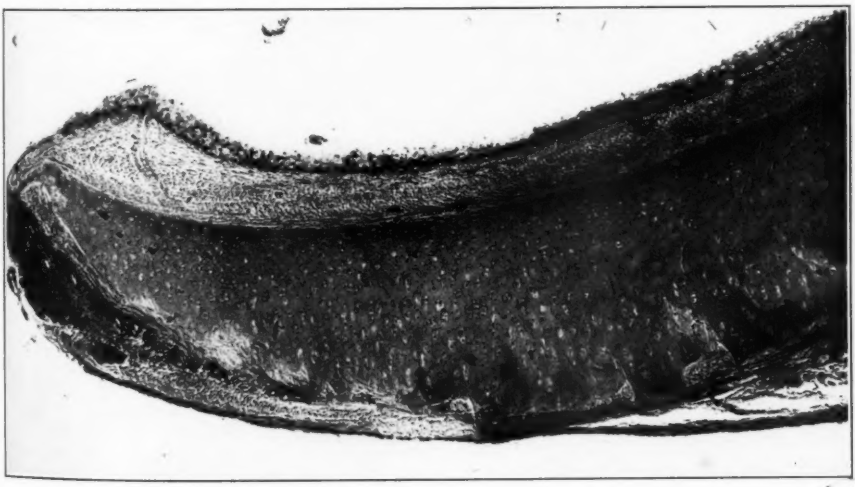

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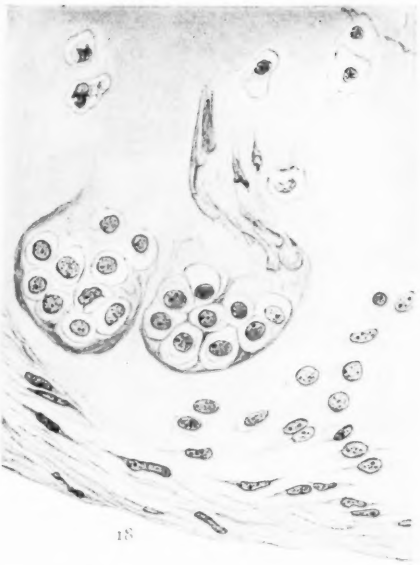
PLATE 96

FIGS. 18, 19, 20, 21. Camera lucida drawings of the reparative changes seen in a defect in the patellar groove after a period of twenty-eight weeks. Note the clusters of cartilage cells within lacunar spaces, the disappearance of some of the lacunar margins and the extension of the cartilage cells from the original hyaline cartilage into recently formed tissue which partially filled the defect crater. Obviously multiplication of cartilage cells within lacunar spaces had occurred although no mitotic figures were found. Fig. 19, × 420; Figs. 18, 20, and 21, × 630.

19



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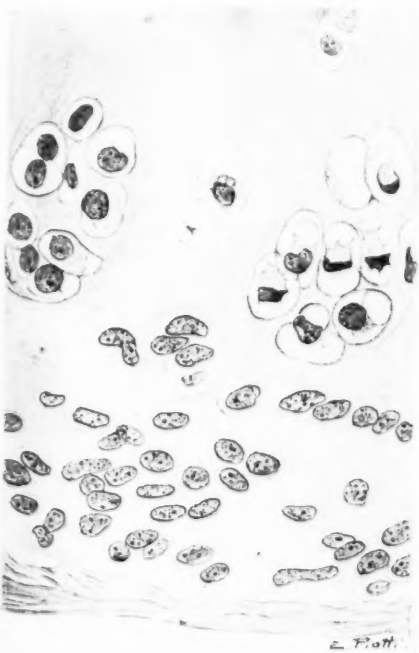

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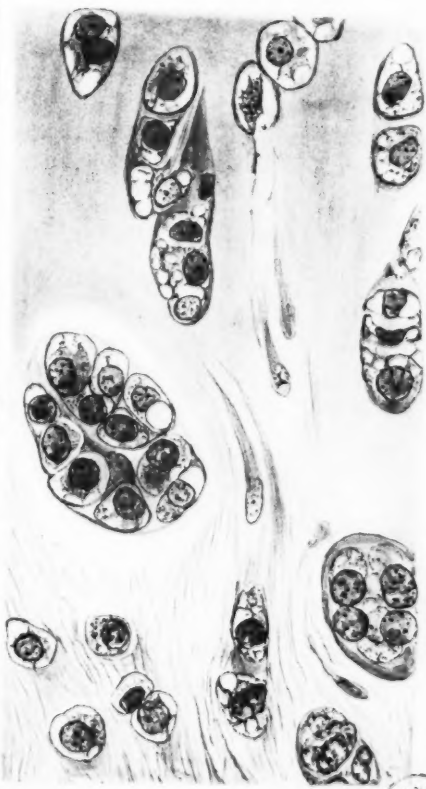

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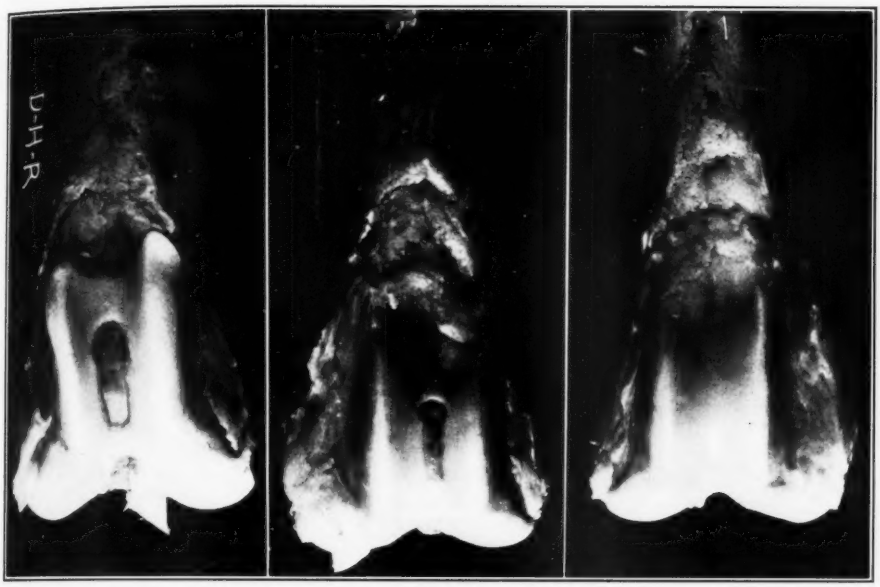
PLATE 97

FIG. 22. Gross photograph (natural size) showing a defect in cartilage and subchondral bone after a period of twelve weeks. No important intra-articular changes had occurred.

FIG. 23. A natural size photograph of the right and left joints showing no important difference between them except for a surgically made defect in the cartilage of the patellar groove of the right joint. The right knee joint was operated upon twelve weeks before, the left joint served as a control.

FIG. 24. A gross photograph which illustrates the marked villous overgrowth of the synovial membrane which occurred in the joints where the patella was dislocated. The surgical procedure in this joint was identical to that used in the joint illustrated in Fig. 22. × 2.5.



22 23a 23b

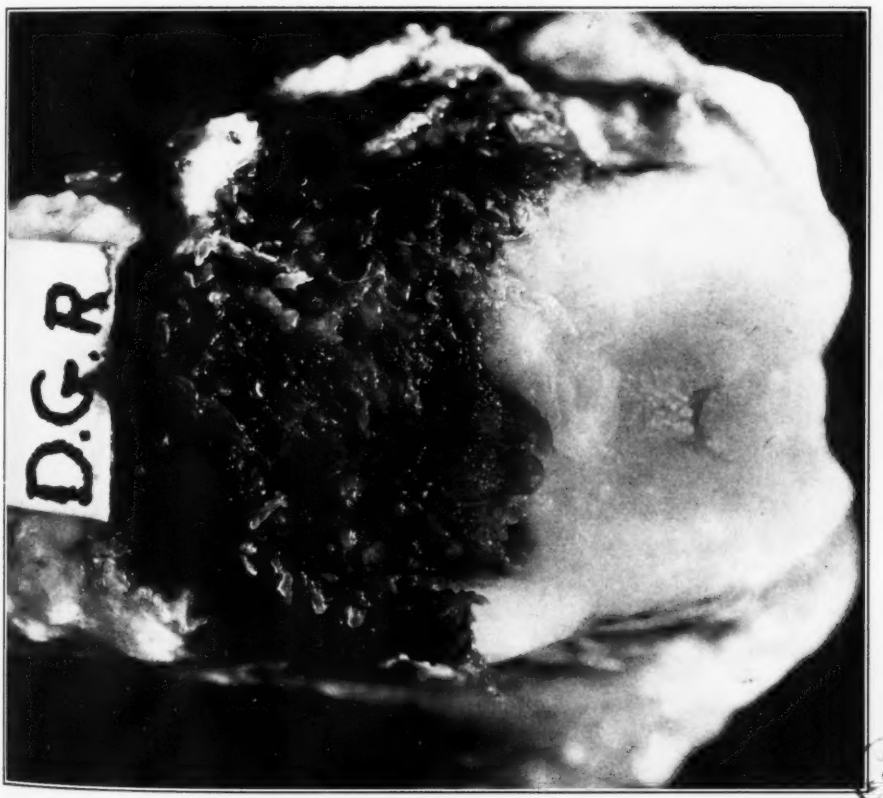


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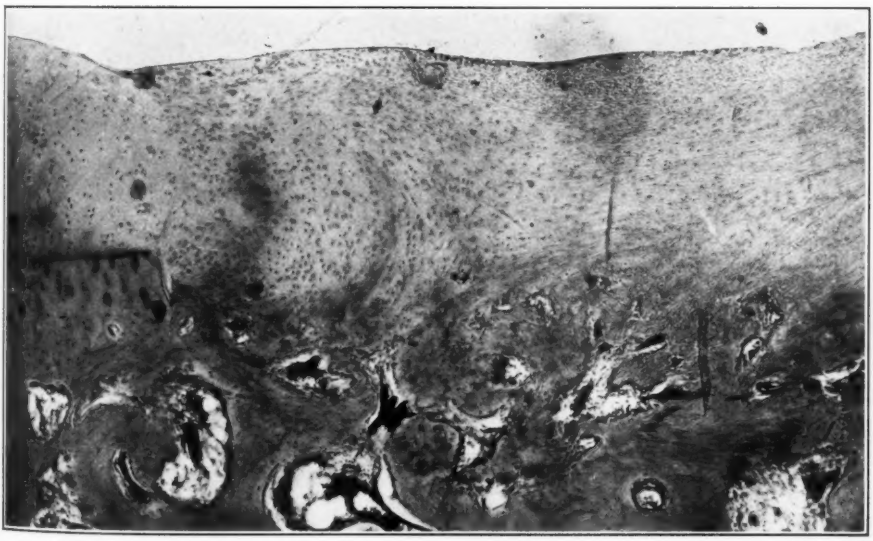
PLATE 98

FIG. 25. A low power photomicrograph of a defect in cartilage and subchondral bone after a period of four weeks. Note the absence of proliferation of bone or cartilage. The crater of the defect is filled with fibrous tissue. × 76.5.

FIG. 26. The repair of a defect in cartilage and subchondral bone after a period of twenty weeks is illustrated in this photomicrograph. The fibrous tissue which was found in earlier specimens (Fig. 25) now resembles fibrocartilage and imperfectly formed hyaline cartilage. The matrix of the recently formed tissue and the original cartilage is fused and new bone has largely filled the defect crater.

25



26

STUDIES IN THE PATHOLOGY OF DEVELOPMENT*

II. SOME ASPECTS OF DEFECTIVE DEVELOPMENT IN THE DORSAL MIDLINE

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INTRODUCTION

Developmental disorders of various kinds are particularly frequent in the midline of the back. In speaking of the dorsal midline we do not restrict the term to a narrow strip in the axis of the body, but have in mind rather a more extensive area, with variable or even indefinite lateral limits, symmetrically located in respect to the sagittal plane, and extending the entire length of the body, through the head and onto the face. As regards the depth of this region, *i. e.*, its dorso-ventral extent, it is possible to be rather more precise. It may be considered as extending from the nervous system outward to the covering integument, including both of these as well as all of the varied structures which have their proper place somewhere in between. The deviations from the normal or usual conditions, which may be encountered in this region, range all the way from the most inconspicuous variations and anomalies to wholesale defects of great extent. Hardly noticeable and of no importance at one end of the series, they appear at the other end as gross and widespread malformations, whose presence makes independent existence quite out of the question.

The peculiarities of the dorsal midline, its great inherent sensitivity and the special developmental risks which are attendant upon its proper formation, have been considered more in detail in a recent article,[1] but a few of the more important points may be noted here again. As a result of the very radical structural alterations, both gross and histological, each with its genetic implications, and of the relatively massive and far-reaching rearrangements of tissues which occur during the formation of the vertebrate nervous system and the restoration of proper continuity over it, there has been impressed

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upon the whole dorsal midline a certain lability or even a vulnerability, which paves the way for all sorts of defects and variations. The nominal risks incurred in these extensive changes must be further increased by the peculiar qualities and capacities of the cell layers primarily involved — the central nervous system and the epithelial covering of the body. Not only this, but it may very well be that in human development the dangers are even more enhanced, since the necessary developmental procedures must be carried out on tissues whose natural sensitivity has been further increased by specifically human capacities and susceptibilities. Although this applies primarily to the central nervous system, it is not without its effects elsewhere in the midline.

We are concerned at present, however, not with the gross and extensive defects which are so often found on some part of the dorsum, but with the milder, much less serious cases, and with these again in their incipient stages, as they appear in the human embryo relatively early in its development. There can be no doubt that many, if not all of these cases, from the severest to the mildest, belong in the same category, as visible expressions of some derangement in the normal and orderly growth and differentiation of the various structures and tissues concerned. The severest cases are obviously those in which the neural tube is wide open and as a result many other structures are or would have been markedly deficient. It is not so easy to say, on the contrary, what would characterize the mildest cases; or at what point the normal might be said to grade into the abnormal. This difficulty is especially apparent later in development and in the adult, where the multiplicity of the structures involved, their complexity and natural variability, render difficult, if not quite impossible, any very accurate grading as to the severity of the damage done, or as to the extent of deviation from what might be considered essentially normal.

As far as the specimens under consideration at present are concerned, one might have anticipated rather less difficulty in recognizing a varying degree or extent of injury to the embryo, but conditions are by no means so evident or uncomplicated. Leaving the nervous system out of account, the structure of the body wall dorsal to it is still, in these cases, exceedingly simple and the opportunities for deviations from the normal would appear to be rather limited. But even here, however, during the first two months of develop-

ment there is not a little variety in the degree and character of the tissue changes in the midline. These vary considerably in location, extent and apparent severity, as well as in the histological pictures presented. For these reasons it is not often possible to distinguish between earlier or later stages of the same process, if such there be; nor can one always be sure that the real damage to the tissues is greater or more serious in one case than in another. It is altogether probable that the slightest degrees of disturbed development do not appear in this series at all. They would be expected in apparently normal embryos, but only further development would reveal the presence and extent of such latent defects. As long as growth and differentiation might continue there would be the opportunity for these weak or submerged influences to manifest their own peculiar character. In the present series, however, only those cases have been included which show definite and undoubted alterations in the embryo, readily discernible on gross examination.

On account of the importance and significance of the central nervous system in the formation and inherent integrity of the dorsum, and on account of the simplicity of the dorsal structures in the earlier stages, it is convenient to classify the various malformations in this region in terms of the condition of the nervous system. In most of the cases to be considered here, and in all of the more typical ones, the nervous system has been properly formed and closed in behind, while the defects which occur involve only the dorsal body wall, connective tissue and covering epithelium. Since these disturbances in development are much less marked and since they appear only after the neural tube has closed, they may be looked upon as relatively mild derangements. It is not implied, however, that the formation and closure of the neural tube guarantees in any way its own subsequent normal development, any more than that the same would hold for the overlying structures. In these typical, milder cases, the nervous system is to all appearances normal and intact, but the overlying parts give unmistakable evidence of being the site of abnormal processes. What continued development would have brought about may be very problematical, but the visible evidences of the damage already done are confined to the parts dorsal to the nervous system, or even to the superficial epithelium.

One cannot escape the conclusion that in the formation of the central nervous system and of the various structures which close it

in dorsally, a very definite developmental risk has been incurred, and that the parts concerned have had impressed upon them a greater degree of sensitivity and vulnerability than is to be found elsewhere. In the great majority of cases development proceeds normally throughout the body, but disturbed or altered environmental conditions may be expected to compromise or upset, more or less seriously, this orderly development, exactly at those points where the cells and tissues are, for some reason, the most sensitive. There are, early in the life history, particularly in man, two especially vulnerable regions of the body: one is the dorsal midline, the other is the distal portion of the extremities. In view of the normal and inherent susceptibility of these parts to unfavorable environmental influences, it is not necessary to predicate any special germinal, genetic defect to account for most of these cases of maldevelopment. It would seem very probable, however, that this same susceptibility might be a favorable or vulnerable point of attack for various influences which would later appear as germinal or hereditary defects. In any case, the ultimate factors involved are essentially germinal, whether they merely determine an unusual, even dangerous sensitivity, or whether they actually allow or lead to gross malformations. If the genetic composition of the future individual prescribes and carries through a certain normal course of development, then it also and just as certainly provides many of the pitfalls along the way.

The individual specimens to be described below have been arranged roughly in order of the degree of disturbance in the dorsal midline, with some regard also for the general condition and quality of the embryo, beginning with those which show the most pronounced defects and terminating with those where the damage done is relatively slight and perhaps of no very great consequence. The purpose of this arrangement is to call attention to, and to contrast with each other, the major and minor defects which occur in this region. On the one hand the developmental disturbance is at a maximum, with extensive involvement of the nervous system; while on the other it is at a minimum, and only slight alterations in the superficial epithelium can be made out. Between these extremes the order is less trustworthy and of less significance, as already indicated.

Description of Specimens

The first few specimens which will be considered have been included here simply as illustrations of typical, major midline defects in the embryo. They stand at one end of the series, which at the other fades out into normal or quasinormal conditions. The series presented here might have been extended, a greater variety of cases might have been introduced and the descriptions and histories could have been more detailed, but we have endeavored to cover the more important points in a reasonable amount of space.

Embryo No. 83: (Fig. 1). No history was available. The embryo is rather pale and chalky in appearance and there is a deep tear ventrally between head and trunk, which exposes the heart. Before being damaged the greatest length would have been approximately 7 mm. The head is rather small and there is little relief or detail to be made out. The cord is small and shows no trace of vessels.

Beginning about the first sacral segment and extending almost to the end of tail, there is a wide-open defect in the neural tube. This defect is perfectly regular in outline, measures 2.5 mm. in length and occupies the prominent convexity of the sacral curve extending over eight to ten segments. It is widest near its anterior limit, a little less than 1 mm., while at the opposite end it is a trifle more than 0.5 mm. in width. There is a distinct, sharp linear groove in the center, which does not quite reach the anterior limit of the defect, due to the rolling in here to form the neural tube. At the caudal end the median groove appears to run out onto the dorsum of the tail, and there is no evidence of any attempt at closure or even of the presence of nervous system beyond this point. The widely everted walls of the neural tube, on either side of the midline, are smooth and convex, no details being visible. Their lateral margins are sharp and even and overhang slightly the adjacent body wall.

Sections of the embryo show that extensive disintegrative changes are well underway. These are most noticeable in the head where the brain has suffered most. In some parts of the nervous system, more especially in the cord, the ventral portions seem rather better preserved and less affected than the dorsal parts, but this might be an indication of better nutritive or circulatory conditions in the former. In spite of the fact that the open neural tube in the sacral region was sharply defined and seemed very well preserved in the gross

specimen, the changes in its walls are more marked than in any other part of the embryo. The form has been preserved but the substance is almost lacking. There has been an extensive cytolysis and the remaining tissue is more like an attenuated mesenchyme than nervous or epithelial tissue. The free, exposed surface is often irregular and indefinite, while the deeper surface seems to merge in many places into the loose underlying mesenchyme.

The vesicle which contained this embryo was rather large, 29 by 27 by 22 mm., the villi were well developed and numerous, but many of them showed early cystic changes. Within the sac there was a large amount of dense, stringy magma which quite obscured the embryo. The amnion was of normal size but much thickened, and presented a large rent through which the embryo had escaped. Except for a few fine tortuous vessels faintly visible on the yolk-sac, there were no vascular channels to be seen anywhere.

Embryo No. 46: (Fig. 2). There was a cessation of menstruation in this case two and a half months before the abortion, but the embryo is much younger than this, for its maximum length is only 14.5 mm. It is markedly stunted and malformed, of a brownish, muddy color, more opaque than normal and has obviously been dead for some time. The body is short and straight, rather cylindrical and gives the impression of being unduly distended. The head is extremely defective, most of the brain seems to be absent, so that the face sets on the anterior part of the trunk with the upper margin of the mouth as the most prominent point. The limbs are fairly well developed although the posterior ones are somewhat retarded. The cord is short and much constricted at its embryonic and chorionic attachments. Between these points it is very greatly dilated, with no evidence of vessels within.

Occupying most of the dorsum of the body and extending forward to within 1 mm. of the eyes is a large pyriform area over which the superficial ectoderm is either lacking or markedly altered. This area is quite symmetrical and measures 9 mm. in length by 6 mm. in width. It is widest in front where it is also raised a little above the general body surface, its limits are quite sharp and even, and it is distinguished from the rest of the embryo by a slight yellowish tinge. In its anterior part this surface is dull and uneven, marked by small, shallow, irregular furrows and depressions or even small cavities. The picture presented is that of a disintegrating surface

tissue, the end results of which are seen in the posterior part of the area. In this later location there is a deep, rather clean defect which was originally filled with prominent, but very irregular masses of degenerating nervous tissue. This tissue was so friable and loosely attached that it was lost in the removal of the embryo from the sac.

Except for the nervous system the body tissues and organs are in better condition than was anticipated. The anterior end of the nervous system shows the characteristic complicated foldings and widespread histolysis and both the pigment and nervous layers of the retina show similar changes. Farther back, as shown in the gross specimen, practically all of the brain and cord has been lost. A study of the sections reveals the fact that the superficial ectoderm is still present over much of the anterior part of the dorsal area which appeared defective upon gross examination. The superficial layers are, as a rule, somewhat thickened, but there are various places where they are quite thin or even entirely wanting. Structurally there is little to be made out definitely, but this region stands in marked contrast with the smooth, even development of the surface epithelium in other parts of the body. It is obvious that the proper development of the ectoderm of the dorsum of the embryo has been radically altered. The original disturbance, whatever it may have been, has not prevented the closure of the neural tube, at least in its anterior part. It has, nevertheless, left its impress on the superficial layers which make up the skin in this region, as shown by its abnormal appearance, both in the gross and microscopic. This compromising of the proper development of the superficial structures in the midline of the back, with little or no involvement of the central nervous system, will be brought out more in detail in the later cases. It is the expression of a greater sensitivity or vulnerability in this region, due primarily to the massive and radical developmental processes which form the central nervous system and the body wall behind it.

The chorionic vesicle is very large, 60 by 40 mm., thin-walled, and shows extensive hemorrhagic areas; most of its surface is covered by thin, adherent decidua and it is filled with a turbid, blood-tinged fluid. The amnion is everywhere in contact with the chorion, but no vessels are to be seen, even in the immediate neighborhood of the embryo. Villi are few in number, small and fibrous.

Embryo No. 129: (Fig. 3). The embryo is represented by a fairly regular, cylindrical mass with rounded ends, 12 mm. in length by 6 mm. in diameter. It is the most stunted and deformed of any of the embryos in this series and shows in addition some of the most interesting surface changes. The color in general is a grayish brown with some darker blotches, while the cord, which comes off the ventral surface at the posterior end of the body, is much lighter. The face, and what remains of the head, make up the anterior hemispherical end of the embryo, the only relief here being found by the wide-open, quadrangular mouth which is completely filled by the tongue. The eyes on both sides show a well defined ring of retinal pigment, with the choroidal fissure pointing toward the mouth.

On the dorsum of the embryo, but much nearer the anterior end, there is a roughly circular, well defined area, which measures about 4.5 mm. in diameter. In spite of the brownish cast and general discoloration of the embryo, this patch, on what should be the vertex or back part of the head, stands out very conspicuously. It is quite symmetrical but a trifle more extensive on the left side along its posterior border. Within this area a little differentiation can be made out, in that the central portion is smoother and rather yellowish in color, while outside of this is a darker, uneven, ragged zone covered over with numerous small appendages or tags of tissue. There is nothing to indicate an open neural tube in this region, the appearance being rather indicative of profound alterations in the superficial tissues.

The sections show that the embryo is much older and more advanced than its gross characters might indicate. The brain and head in general are very defective. The cartilaginous skeleton of the vertebral column and of the larger bones of the extremities is very well defined, but there is a very marked kyphosis, the anterior end of the spine being at right angles to the more posterior portions. The superficial tissues of this embryo, especially in the dorsal area noted above, present a most bizarre and unusual appearance. No attempt will be made at this time to describe in detail these conditions which appear quite foreign and out of place in an embryo of this stage of development. While much of the superficial ectoderm, and in varying extent the underlying tissue also, are very radically altered, the greatest degree of histological distortion and maldevelopment is found in the circumscribed area on the back. There is

here an extensive but very irregular thickening of the surface layers, and also what may be called provisionally a widespread hyperkeratosis, with a varying amount of desquamation and the formation of structures which may be characterized as epithelial pearls. The connective tissue beneath is often thick and dense and there are large spaces or clefts, but their relation to epithelium or connective tissue is not always clear. In a few scattered areas there are very definite pigment cells, usually occurring in small clumps. One of the most remarkable features in this specimen is the contrast presented in different tissues in regard to their preservation and in the evidences of continued cell life and activity. Although practically all of the embryo shows advanced histolysis and general disintegration, with the exception of the cartilaginous elements, there are certain regions on the dorsum where the subepithelial structures appear to be made up of perfectly normal healthy cells. It is here that one encounters a rather dense connective tissue, with groups of cells of doubtful significance which often contain conspicuous masses of pigment. The staining quality of the tissues in these regions is quite satisfactory, while everywhere else it is very poor if not entirely wanting. Although the embryo as a whole and almost all of its constituent cells have long since been dead, the cells in the areas just noted seem to have been living practically up to the time of fixation. They form an oasis, as it were, in the desert of death and dissolution around them. They are also peculiar in that they seem to represent a stage in development much in advance of the possible chronological age of the embryo. In this precocity, if the term is permissible, there may be some pathological tendencies, and the same would apply to the hyperkeratosis, so-called, in the overlying ectoderm. The general picture is that of a histological differentiation far in advance of what it should be, a remarkable form of prosoplasia.

The chorionic vesicle is very large, quite out of proportion to the embryo, measuring about 60 by 100 mm. It is invested everywhere by a thick layer of clotted blood. Its internal surface is rough, uneven and dark in color, due to the extensive clots without. The amnion and chorion are fused. The villi are not very numerous, they are thick and fibrous and there is considerable leucocytic infiltration in the surrounding blood. No vascular connections between embryo and chorion are visible.

Embryo No. 665: This specimen is from a first pregnancy at the age of 17 years. There is a history of pernicious vomiting. The menstrual history is somewhat uncertain, but the last period was at least two months before abortion. On account of the damage to the posterior end of the body, the original length cannot be determined, but it was probably not far from 15 mm. The head is small and the details of the face much obscured. Over the anterior end of the head and the upper part of the surface ectoderm is lacking. This defective area is rather extensive and quite symmetrical; it is bounded by a line which runs from about the angle of the mouth upward and backward across the eyes, reaching the midline behind, not far from or just beyond the midbrain. The surface of this area is rather more brownish in color and along its margins the adjacent body epithelium appears loose and slightly elevated. In the midline behind the large superficial defect, in what seems to be the region of the lower rhombencephalon, there is a small, elongated, deep-seated, apparently hemorrhagic spot about 0.5 mm. in length.

Although the ventral thoracic and abdominal walls are torn away, exposing the heart, there are some evidences that the anterior body wall might have been defective over the upper part of the heart, an incipient ectopia cordis: there is also a brownish discoloration here similar to that noted in the head region.

Histological examination reveals a beginning dissociation of the tissues, most noticeable in the nervous system, but the staining reactions are still fairly well preserved. The large defect is seen to be quite devoid of epithelial covering, but along its margins the body ectoderm stops very abruptly and is often heaped up into large prominent cell masses. The cells which make up these conspicuous masses are large and pale, irregularly polyhedral in shape, often several layers deep and seen to be derivatives of, or correspond with, the superficial periderm. There are indications of a similar, but less conspicuous, heaping up of cells along the border of the ventral tear, or the defect over the heart. The surface of the large defect on the head is smooth and even, and the limiting connective tissue cells often appear as a thin, well defined layer of squamous cells. Scattered among the superficial connective tissue cells there are considerable numbers of fairly large, roughly rounded, epithelioid looking cells, with small dark nuclei and of a peculiar brownish pigment,

but the coloring material is very finely and evenly distributed throughout the whole cell and there are no indications of the usual pigment granules. These cells may account, in part at least, for the darker color of this part of the embryo.

Embryo No. 536: Only the embryo was obtained in this case, which represents the sixth pregnancy in a woman 30 years of age. The first pregnancy went to eight months, and this was followed by four miscarriages, from the fifth to the seventh months. The miscarriage in this last pregnancy occurred six or six and a half months after the last period. During the latter half of this pregnancy the patient was in poor condition, bad tonsils and infected teeth are noted in the history, also the possibility of lues. The placenta was said to be 50 mm. in diameter and to be markedly necrotic.

The greatest length of the embryo is 17.5 mm., its color is poor and there is some shrinkage, the superficial layers show numerous fine wrinkles and seem to be desquamating in many places. The head is rather small. In the sacral and lower lumbar region behind, there is a large, fairly regular area, somewhat more brownish in color, over which the superficial ectoderm seems to be missing. This area is very slightly depressed and its posterior margins are rather sharper and more symmetrical than the anterior. It measures about 4 mm. in length by 3 mm. in width.

Sections through the embryo show the usual dissociation and lack of staining qualities. The brain is much more involved than the cord — even its major subdivision can hardly be recognized. The epidermis varies considerably in thickness and structure in different regions, but it is often made up of several much flattened stratified layers which show a marked tendency to separate from each other and also from the connective tissue below. There is widespread desquamation of the more superficial cells, while much of the epidermis gives the impression of being made up of stiff, hard cells, a condition in some ways not unlike those observed in Embryo No. 129, where there seemed to be a hyperkeratosis. In a few places, on the dorsum anterior to the defect, there are small "epithelial pearls" embedded in the epidermis. In addition, there are small scattered groups of cells which lie close to, or in contact with the deep surface of the epidermis, and in a few instances they seem to have been derived from the adjacent ectoderm. As noted in other cases, it is not always possible to identify the exact limits of the de-

fect on microscopic examination, although in the gross specimen these were often quite definite and conspicuous. In some of these embryos the surface layers stain very intensely with hematoxylin, while the deeper structures may be quite unaffected. In this particular case the staining is especially intense in the general region of the defect and although structural details cannot be made out, there appears to be no very striking difference between the epidermis here and elsewhere on the body. That the surface epithelium of the back, particularly lower down, has been affected more, or in some other way, than the same layer of cells elsewhere, is indicated by the presence here of subepithelial groups of cells, of epithelial pearls and possibly also by the somewhat different staining reactions. In certain localities also there seem to be considerable amounts of pigment in the epithelium, but it is so masked by the stain that its presence is at least doubtful. Over most of the sacral defect the surface epithelium is quite intact, but near the end of the cord there are several places where it is lacking.

Embryo No. 161: (Fig. 4). No history accompanied this specimen. The embryo is in very poor condition, the head being almost completely detached from the body, the greatest length not far from 12 mm. The head appears small, particularly its anterior end, and in the face only the eyes can be distinguished. On the dorsum of the head, about in the region of the anterior part of the rhombencephalon, there is a transversely elongated, somewhat elevated, uneven mass which measures 3.5 mm. from side to side and 2.5 mm. from before backward. There is nothing to be made out on the surface and the color is substantially like that of the rest of the embryo. The outlines of this dorsal patch are quite definite and it is also fairly symmetrical. Sections through the head of the embryo show practically nothing that can be identified as the area seen in the gross in the back of the head. There is extensive dissociation everywhere although the nuclei still stain fairly well. The superficial ectoderm is very thin and for the most part quite inconspicuous.

The chorionic vesicle is of moderate dimensions, 33 by 26 mm., but it is thin and translucent and there are only a very few long, stringy villi. These villi lie close against the vesicle wall and are all directed the same way, as if smoothed out by some slipping or dislocation of the vesicle. The amnion is loosely fused with the chorion and its cavity contains a large amount of light flocculent precipitate.

The distal half of the cord is much smaller than the proximal part; no vessels can be seen anywhere.

Embryo No. 210: (Fig. 5). In this case there had been eleven previous pregnancies, nine births at term and two miscarriages at the end of the first month. The mother was 40 years old and there was a menstrual history of fifty-one days. No cause was given for the abortion. The embryo, whose greatest length is 15 mm., is considerably deformed, the head is small and the face appears to be fused with the ventral surface of the trunk. The posterior end of the body is small and tapering, the extremities are somewhat retarded. The cord is short and straight, much distended and shows a small, pedunculated appendage near the embryo. In the dorsal midline, over the rhombencephalon there is a large thin-walled bleb, 3 mm. in diameter and elevated about 1 mm. above the surrounding surface of the body. The limiting walls of the bleb are rather steep and slightly undercut where they join the superficial ectoderm. No gross defects are to be seen around or beneath the superficial bleb and the surface layers of the body are elsewhere unaltered.

Although the embryo is in poor condition histologically, the dissociation of tissues is more marked in the region of the head and face than it is farther back, the brain having suffered most severely. Throughout the cord the dorsal half is beginning to break up, but the ventral portion is in much better condition. No definite changes of any kind can be seen in sections through the dorsal bleb. Both the superficial ectoderm and the underlying structures appear unchanged, except for their separation and the irregular foldings in the surface layer. The developmental damage in this instance is relatively slight, manifesting itself simply as an accumulation of fluid underneath the ectoderm. There can be no doubt that even milder disturbances may occur here, as well as elsewhere, but they are latent, in a sense, and become conspicuous or recognizable only later in development.

The vesicle belonging to this embryo is substantially normal as regards its size, but its walls are thin, the villi very poorly developed and there is considerable hemorrhage under the decidual layer, by which it is completely surrounded. No vessels can be seen either in the sac or in the embryo.

Embryo No. 597 B: (Fig. 6). This specimen, from a woman 37 years of age, is the smaller of two fraternal twins. There had been

two previous pregnancies, the first going the term four years before, while the second terminated in abortion at about two months, possibly due to a fall. The present, or third, pregnancy also resulted in abortion, some eleven months after the preceding one. The last menstrual period began fourteen weeks before the abortion, but there is a history of menstrual irregularity for the two months preceding the last period, and of occasional morning sickness for two months before the abortion.

Judging from its condition the smaller fetus has been dead for some time, its larger companion is in very good condition but it is slightly distorted and has suffered a little from drying. The larger one is four times as long as the smaller, the sitting heights being 130 and 32.5 mm. respectively. The placenta and membranes of the larger one were not received. The smaller of the twins (No. 597 B), is light yellowish brown in color and the most superficial layers of cells are desquamating in shreds and sheets of considerable size. On both hands, the fingers, which are short and apparently fused together, are encased in what appears to be a much thickened epidermis. The feet seem to be normal, but the legs show a marked ventral convexity and the thighs appear rather short. In the midline over, or just behind, the vertex of the head there is a very conspicuous, symmetrical bleb which has an anteroposterior extent of 7 mm. It is translucent and the regular outline of the head can be made out beneath it. The deeper structures are not involved. The cord is very much kinked and twisted, it varies considerably in size in different places, and obviously there has been no circulation through it for some time. Arising from the cord, close to its attachment to the fetus, are several large, thin-walled, almost pedunculated blebs or vesicles.

The placenta is quite out of proportion to the fetus, measuring 100 by 70 mm. Its fetal surface is irregular and nodular in appearance, due to the extensive subchorial hemorrhages. The amniotic fluid was turbid and discolored.

Embryo No. 407: (Fig. 7). This is a rather typical stunted embryo in a large vesicle, with a menstrual age of nearly ten weeks. It was the first pregnancy and came from a woman 38 years old. For two days previous to the abortion there had been bleeding and abdominal pain. The embryo measures 7 mm. crown rump. It is very pale and of quite uniform color throughout. The head is very

small, there are no indications of eyes, and only the first branchial arch can be made out. The limb-buds are small but fairly well developed, segmentation is only faintly indicated. Immediately above the anterior extremities there is a large bleb-like swelling on either side. In the dorsal midline, in the lower thoracic region, there is also a conspicuous elevation of the superficial layers over a small circumscribed area. Farther back, opposite the posterior extremities, there is a longer, but much less conspicuous swelling, not noticeable in the illustration.

The tissues of the embryo are in very poor condition, the nervous system, and more particularly its anterior part, being most severely affected. In the region of the superficial changes noted above, the constituent tissues are apparently unaltered, save for the separation of the epithelium from the underlying connective tissue, and the tearing and displacement of the former. All of the structures stain very poorly.

The sac is somewhat distorted and thin-walled. It measures 52 by 35 by 20 mm. It is very pale, practically free from blood and almost completely covered by thin, smooth decidua. Where exposed, the villi are rather slender and scattered but not especially abnormal. The amnion is large and somewhat thickened, and through a rent in it the embryo had escaped into the exocoelom. Blood vessels are not to be seen either in the embryo or in the membranes.

Embryo No. 442: (Fig. 8). The specimen to be described here is a tubal pregnancy from a colored woman 26 years old. Four years ago there had been an abortion at three months, the cause being undetermined. For several years there had been a bilateral salpingitis, also dysuria and frequency of urination for the past three months. The last menstrual period was ten weeks before operation, but throughout most of this time there had been spotting and pain in the left lower quadrant. At operation there was a chronic salpingitis on the right side, the uterus was small and forward and there were simple cysts in both ovaries. No mention is made of a corpus luteum.

Within the tube is a turbid amber fluid, apparently blood-stained. The embryo, which has a greatest length of 26 mm., appears fairly normal, although its color is not good. The eyes are large and conspicuous but the lids appear retarded in their development, due possibly to mechanical interference from the skin defect over the

forehead. Below and internal to the right eye, there is a large pit-like defect.

In the midline behind, in the lower dorsal and lumbar regions, there is a slightly elongated area, about 7 mm. in length, over which the superficial layers seem to be wanting. This area is, in general, quite symmetrical, but a trifle more extensive on the right side. The right border is especially conspicuous, appearing as an irregular, ragged, elevated line of thickened or partially detached epithelium. In the anterior part of this area there is a smaller, darker patch, quite sharply marked off and situated almost exactly in the median line. On the anterior surface of the head, a short distance abcve the eyes, there is a large and very conspicuous, brownish discolored band, extending almost the entire width of the head. This frontal patch is much more striking in appearance than the one on the dorsum; it is also a little darker in color and more abruptly set off from its surroundings.

The cord is small, short and straight, its distal two-thirds is occupied by a large thin-walled, spindle-shaped enlargement, but at its attachment to both embryo and chorion it is very much constricted. No definite blood-containing vessels can be seen within it.

Sections through the posterior part of the embryo show that over most of the dorsal area noted above, the surface epithelium is intact. It stains very densely so that its structure cannot be determined, but it seems to be rather thicker than elsewhere. Along the margins of the area, however, there are numerous conspicuous thickenings in the ectoderm, as can be seen in Fig. 8. These thickenings are as a rule small and scattered, and they vary much in form and size from broad low mounds of cells, to slender, almost pedunculated outgrowths. The cells appear as if heaped up on the surface, without any involvement of the deeper layers. In general the subepithelial tissue seems rather denser and more fibrous on the back of the embryo, but it does not present the marked alterations to be seen in the frontal region. In the smaller central patch on the back the epithelium is definitely absent, and along its borders the limiting epithelium is thickened and ends abruptly, but the large, exuberant masses which are present farther back and more laterally are not seen here. Not only are the surface cells lacking here, but there is further evidence of disturbed development in the presence of numbers of large, rather wide clefts in the exposed connective tissue.

In addition to the spaces in the connective tissue of the dorsum there has been an extensive accumulation of fluid behind and on either side of the posterior end of the spinal cord, so that the latter appears to lie on the ventral wall of the large open cavity. At the very extremity of the cord there is a small irregular diverticulum which comes off from the ventral part of the central canal.

No essential difference can be seen in the epithelium over the frontal discoloration, as compared with that of the dorsal area, but in both cases the details are obscured by the intense staining. The former, however, shows only a very slight thickening of the ectoderm in a few places near the margins of the area. In the underlying tissues, on the contrary, there is a very striking difference. Running through the more superficial part of the sub-epithelial connective tissue there is what appears to be a rather denser stratum of the same tissue, but one which is stained almost as deeply as the ectoderm outside. This stratum is sharply delimited, near the center of the area it lies very close, if not in contact with the epithelium, but elsewhere there is interposed a thin layer of paler, normal connective tissue. Apparently this stratum was responsible for the very obvious and well circumscribed discoloration seen on the forehead in the gross specimen.

Embryo No. 652: (Fig. 9). No menstrual history was obtained for this specimen, which came from a fibroid uterus. Even when fresh the condition of the embryo was not good, the color being a turbid brown, the greatest length a little over 15 mm. There seems to be some desquamation of the surface epithelium, but this may be in part adherent precipitate. The head, especially its anterior part, is small, the mouth is widely open and its lateral angles may be torn somewhat. Trunk and extremities are fairly normal, although the latter appear slightly retarded.

On the dorsum of the head, over the anterior part of the rhombencephalon, there is a rather definite pale orange discoloration. It is symmetrically disposed as a transverse band which is best developed on the right side and somewhat indistinct in the midline. As far as can be seen the surface epithelium is intact and not materially altered.

The cord, which is small and straight, shows a small but prominent bleb close to the embryo. There is little evidence of vessels within the cord.

Although the tissues of the embryo are beginning to dissociate they still stain fairly well. The epithelium over the dorsum of the head is everywhere intact and shows no definite alterations in the arrangement or in the general morphology of its constituent elements. The discolored area seen in the gross specimen is, however, quite recognizable. It appears under low power, stained with eosin, as a fairly well circumscribed, pale yellowish pink region in the superficial ectoderm. There is not the even yellowish tinge seen in some of the connective tissue cells, in Embryo No. 665, or the very evident pigment granules which were present in Embryo No. 129. It looks more as if the region in question had been dusted over with a fine powdery substance, which, although it seems to have no definite relation to the epithelial cells, is confined to them and is not to be seen in the underlying tissues. This material is quite evenly distributed and its limits are rather definite. Scattered through these pinkish areas there are a few small, rather densely staining nuclei, much like those in the deeper tissues, but more numerous here than in the epithelium elsewhere. This peculiar material does not suggest pigment at all; it gives rather the impression of something which had been applied to the cells or even to the sections. The neighboring mesenchyme does not appear to be in any way involved.

The vesicle is essentially normal, the villi are numerous and well developed; the amnion may be slightly thickened and in the exocoelom there is abundant, coarse, stringy magma.

Embryo No. 167: (Fig. 10). Except for a menstrual history of "about ten weeks," and that the abortion was "not induced," there were no data available on this specimen. The embryo is damaged somewhat, especially about the mouth and upper part of the trunk, and its color is decidedly poor. Its greatest length is 18.5 mm. The head, particularly its anterior end, is small, the mouth is wide open and there are deep tears on either side. Between the eyes are a number of small holes, or pit-like defects. The retinal pigment is paler than usual, and appears red rather than black. Both the anterior and posterior extremities, but particularly the former, are much retarded.

In the dorsal midline, about the region of the lower rhombencephalon, there is a small, transversely elongated, somewhat smoother, discolored area, slightly greenish in color. It is roughly

reniform in outline, sharply marked off from the surrounding parts, slightly elevated and measures about 3 by 1 mm. It does not appear to be a simple stain or discoloration and there is no evidence of any injury or defect. Farther forward, over the posterior part of the forebrain, not shown in the illustration, there is a smaller, more yellowish spot, irregular in outline and measuring about 2 mm. in diameter.

A little in advance of this second patch there are still two others of much the same character. These last mentioned, most anterior spots, lie close together, almost in the midline, high up on the forehead. They are rather darker in color, and even more conspicuous than the large area behind.

There is a most striking similarity to be found in the dorsal area of this embryo and the condition noted in Embryo No. 652. In both cases there is a very definite, transversely elongated, more or less discolored area, symmetrically disposed, exactly in the midline and lying over the rhombencephalon. In Embryo No. 167 the area is widest in the median line, while in No. 652 it is narrower there, and rather bilobed in appearance; in the former the area is a little farther back and it is also somewhat more conspicuous. Not only this, but the histological picture seems, with minor exceptions, to be essentially the same in both cases, the chief, and perhaps only, difference being that in No. 167 the histological changes are more marked and also more extensive. There is, in this embryo, the same powdery, or finely granular material which was found in No. 652. It is perhaps a little coarser, it stains more intensely and has more of a purple or violet color. This difference in appearance may be due in part to the larger quantities of the material present and to the fact that all of the tissues are stained more deeply with hematoxylin than in the other embryo. Not only is this material much more abundant and more closely packed, but most of it is found in the connective tissue immediately below the epithelium, whereas in the earlier case it was confined to the epithelium. In the present embryo it is likewise present in the epithelial cells, but it is much more marked in the underlying tissue. In its distribution in this case it seems much more sharply circumscribed, due apparently to the larger and denser masses involved. The three areas over the forebrain show the same features — if anything the involvement of the mesenchyme is even more marked. Here, as in the previous case,

the epithelium is intact, but there are a few small thickenings around the margins of the area, especially the dorsal area. Over the frontal areas the epithelium appears quite unchanged, but at one or two points there are slight defects, apparently postmortem tears.

The vesicle of No. 167 is of normal size, but it is very thin and villi are practically absent, except for one large clump where they are numerous and thickly set. The villi present are long and somewhat swollen, but there are no globular forms. No vessels can be seen anywhere.

Embryo No. 404: Although this is a tubal pregnancy, the embryo is in much better condition than most of the specimens in this series. Its greatest length is 21.5 mm. and, except as noted below, it appears perfectly normal. This was the third pregnancy in a woman of 27 years. There was nothing out of the ordinary in the first two. The last period was sixty-two days before operation and there had been acute pains in the right lower quadrant for a month. The embryo and amnion were all that were obtained.

The only point to be noted concerns the very posterior end of the body. Here, in the midline of the back, over the lower lumbar and sacral regions, there is an area about 6 mm. long and 2 mm. wide reaching almost to the end of the tail, over which the surface epithelium is apparently missing. The margins of this defect are quite smooth and regular, both its anterior and posterior limits are symmetrically rounded, and there still seems to be some tissue covering the cord. It does not seem possible that this loss of tissue could be due to simple mechanical violence. In the sections there is little to be seen except the absence of the surface cells in the region of the defect. There is some suggestion of a thickening in the epithelium along the margins, but nothing very definite. The exposed connective tissue is unaltered.

Embryo No. 671: (Fig. 11). There are no data on this specimen, except that the abortion was probably self-induced. The embryo, which has a greatest length of 25 mm., is well developed and in fair condition, but there are accumulations of fluid under the epidermis and a number of superficial hemorrhages. Over the posterior aspect of the neck and lower part of the head there is a very extensive area in which the superficial layers are raised up in an enormous bleb. This region is fairly well circumscribed and quite symmetrical. The outlines of the underlying structures, which appear normal, can be

made out quite readily through the thin walls of the bleb. On the left side there is a sharply circumscribed mass of blood covering most of the lateral wall of the bleb on the inside. It appears to have simply settled down into this position, due to the embryo lying on the left side. There is widespread hemorrhage in the superficial tissues on the right side of the face and lower part of the head and a few smaller hemorrhages on the left side. Two or three small, deep-seated hemorrhagic spots can be seen within the bleb, which may be the source of the blood clot on the left side. In the lower thoracic and upper lumbar regions there is a separation of the superficial layers over a considerable extent in the midline, but this is much less conspicuous than the conditions just noted and there is no extravasation of blood. Both upper extremities show considerable vascular engorgement and some actual hemorrhage, especially on the right side.

The chorionic vesicle is rather large for the embryo, and is covered practically everywhere with long, thick-set villi. It is not entirely normal, however, since many of the villi are swollen and irregular, and smaller cystic forms are quite plentiful; the vessels within are quite conspicuous.

Embryo No. 513: Like Embryo No. 404 this is also a tubal pregnancy, but it was the first pregnancy in a woman of 32 who had been married for ten years.

Although this embryo is essentially normal it is not in quite so good condition as No. 404. Its greatest length is 18.5 mm. In the midline behind, just below the fourth ventricle, there is a small oval patch about 4 mm. from side to side and 3 mm. in its anteroposterior dimension. It is not exactly in the midline, but slightly to the right, and obviously the surface layers have been torn away.

From the sections one might conclude that the defect on the back of the head is simply a local accident in a process which is much more widespread. This more extensive condition appears as a marked edema in the subepithelial connective tissue. It is peculiar, in that it is quite symmetrical on the two sides of the body and over the dorsum, and also because the great enlargement of the connective tissue spaces is confined to the more central layers of the mesenchyme, the more superficial as well as the deeper layers being unaffected. The later or final stages of the process are represented by the tearing apart of the much attenuated mesenchyme and the

confluence of the spaces thus formed. This is what has evidently happened on the dorsum where there is a very extensive undermining of the surface layers. At the site of the defect, the outer wall of this fluid-filled cavity has given way, or been torn, and in addition some of it is actually missing. This anasarcous condition extends from the posterior part of the head downward along the sides of the neck and behind the shoulders into the lateral body walls. It encroaches only slightly upon the face in front of the ear. The dorsal midline is not involved, except in the region of the head. The connective tissue and covering epithelium show no changes except the stretching and distortion in the former. It should be noted that the condition is most marked over the dorsum, that it is here only that tearing or loss of tissue has occurred, and also that in the other embryos which are affected in this way it is only the back that is involved.

The vesicle measures 30 by 28 mm., not including the villi. It is somewhat shrunken and pale brownish yellow in color. About half of its surface is covered by long, close-set villi, while the remainder is almost bare. The villi are not normal. Many of them are enlarged and swollen, while many show fine threads like branchings. Bulbous and globular enlargements are common, as well as great numbers of fine, short, side branches. The vessels in the larger villi are more conspicuous than usual. The interior of the sac appears normal.

Embryo No. 611: (Fig. 12). This specimen came from a hysterotomy ten weeks after the last period in a woman 31 years old. It was the second pregnancy and was indicated on account of pelvic deformity which had caused considerable difficulty at the time of the first labor. There had been slight nausea for three weeks preceding the operation. The left tube was found markedly adherent to the lateral pelvic walls and there were three cysts attached to its fimbriated end.

The embryo, which measures 23 mm. greatest length, is apparently normal, although its condition is not quite what it might be. There are only a few points which need to be noted. In the midline, over the cerebellum, there is a minute, thin-walled bleb, except for which the entire dorsum is intact and normal. There are some small scattered ecchymoses behind the left ear, on the left shoulder and on the lateral thoracic wall behind. As seen in ventral view there is

a fullness about the head, behind and below the ears, suggestive of edema.

From the sections it can be seen that the embryo is not as normal as it appeared to be. In the region of the bleb shown in the illustration, the epithelium is separated from the underlying tissue. Farther forward, however, there is another, even more extensive area in the midline where there is an accumulation of fluid, but in this case it is located deeper, within the connective tissue. In addition to these median spaces there are two wide clefts on either side of the midline over the forebrain. Along the lateral aspects of the brain, farther back, the connective tissue spaces are very much dilated and in many places there are wide-open spaces, the condition being much like that seen in Embryo No. 513, but not as severe. Except for these spaces the tissues appear normal, but most of the blood vessels are very much engorged with blood, even the smallest ones.

The vesicle is rather small and is thickly covered with long, richly branched villi which are often matted together. Most of the villi are swollen and irregularly dilated, but the changes are not as marked as in No. 513.

Embryo No. 682: The present series of cases may be appropriately brought to a close with this specimen. The history is somewhat uncertain, but this appears to have been the first pregnancy. The menstrual age is given as two and a half months and the abortion might have been induced. The color and condition of the embryo are not especially good. The greatest length of the embryo is somewhat uncertain but may be taken as not far from 15 mm. The anterior end of the head seems rather small, the mouth is more widely open than usual. Although blood vessels can be seen in the cord, they are hardly distinguishable at its attachment to the membranes. The cord itself is smaller than normal, and most of it is occupied by a large, irregular bleb. On the dorsal surface of the left foot-plate there is an extensive, diffuse, but rather mild hemorrhage.

Exactly in the midline of the back, in the slight concavity behind the midbrain, just in front of the cerebellum, there is a faint brownish, linear discoloration, at right angles to the median plane and not over 1.5 mm. in length. It appears as a narrow, slightly irregular, pigmented line, but its exact nature cannot be made out. Attempts to photograph this dorsal patch were unsuccessful; not only this, but later examination of the specimen, in the gross, showed little or

nothing which could be positively identified as the fine line seen at an earlier date. This is the smallest and least noticeable alteration in the midline that we have encountered in any of our cases.

The chorionic vesicle is of fair size, 45 by 26 by 20 mm. Its walls are thin and the villi, which are few in number, are scattered along one side and at one end. Many of the villi are slender and stringy, while others are definitely dilated and bulbous. The amnion is already fused with the chorion.

Discussion

From the foregoing descriptions of seventeen specimens, it is evident that peculiar conditions obtain in the dorsal midline. Early development may be compromised or deranged in a variety of ways, but the effects are most often in evidence in that part of the body from which is formed the central nervous system. Milder derangements may show themselves only at a later date, or only in those structures which cover in the neural tube behind. The extreme susceptibility of the early nervous system to unfavorable influences, whether occurring in nature or under experimental conditions, has long been recognized, while its significance in human development has been abundantly illustrated by Mall,[2,3] Mall and Meyer,[4] as well as by many other writers. Much less attention, however, has been given to the comparatively slight defects which abound in this region. From our own experience these milder cases may be more common than the more severe ones, particularly during the first two months of development. The possible significance at the end of development of some of these less conspicuous deviations from the normal will be considered on another occasion, likewise the finer histological details exhibited by the tissues affected.

The extensive works of Mall and Meyer, referred to above, contain numerous examples of defective midline development. They also bring out very clearly the great vulnerability of this region, as shown by the high percentage of cases in which it is mainly or alone involved. While our own series has been selected on the basis of minor defects, and for these only, if they occur in the midline, all sorts and degrees of maldevelopment, regardless of their character or location, are included in Mall's and Meyer's material. For this reason many, perhaps the majority, of their cases of midline defects represent severer degrees of damage than are encountered in our

present series. There are, however, in this Carnegie material, a considerable number of cases in which the tissue alterations seem to correspond more or less closely with conditions as we have found them. There is frequent reference to blebs and blisters, loss of superficial epithelium, thickening of the connective tissues, abnormal pigmentation, or the presence of papillomatous outgrowths. Mall speaks repeatedly of ulcerations, particularly on some part of the head, but just how these "ulcers" differ from other defects, which may have a similar location, is not always clear. But neither Mall nor Meyer was especially concerned with any particular type of malformation; the primary object of their investigations was of a more general nature, while the amount and variety of the material to be considered made all but impracticable any detailed account of the findings in each individual case. We cannot be sure, therefore, to what extent the anomalous histological conditions which we have encountered in our material might be duplicated in their specimens. Neither is it always apparent that the defects which they describe are as conspicuous in the gross specimen, as sharply delimited and symmetrical, or as exactly located in the midline as many of those which we have found. Certainly a number of our cases are very similar, even in the gross. Mall [3] (Fig. 6b) shows an unusually large and sharply defined bleb on the back of the neck of Embryo No. 1523, while Embryo No. 2261 (Fig. 72) in Plate 5, Mall and Meyer,[4] is almost a replica of our Embryo No. 671.

Although it is possible to say why the dorsal midline should show a special predisposition to defective development, it is not so easy to say why some of the minor defects should show a predilection for a particular part of the dorsum. It would appear, however, from our own cases as well as from the Carnegie material, that the back of the head, perhaps more exactly the region over the anterior rhombencephalon and midbrain, is more often the seat of defects, usually slight, than any other part of the back of the head. It can hardly be argued that this part is more exposed to external influences than any other. It is more likely that the real explanation is to be found in the inherent factors which govern the growth and differentiation of the human brain and of the tissues which surround it.

We have been interested in these minor defects, partly on account of their relative frequency, but mainly because of the role

they might play in those cases where development is *not* interrupted. It is, of course, not possible to say just what might have been the final result in any particular case. In a few instances one can recognize what may be earlier and later stages of the same condition, but in the individual case there is nothing to indicate whether the process is progressive or regressive or at a standstill. Where the whole embryo is markedly pathological, moribund or even dead, one might suppose that the process would be progressive and terminate only with the death of the cells or tissues involved. In the more normal embryos, however, it would not seem possible to make any prediction, since the greater vitality and growth capacity of the cells might bring about more or less perfect healing on the one hand, or a more pronounced reaction to the causative agent on the other. In some cases the damage seems to be very slight and complete restitution might have been possible, while in others the later stages might have shown local cutaneous defects, or even more deep seated disturbances. Certain aspects of this question will be taken up in a subsequent paper.

In the often sharply located disturbances seen in our specimens there is something akin to the "focal deficiencies" which Streeter [5] has shown to be of such importance in the development of the extremities. Although the predisposing factors may be different in the two sets of cases, it is quite possible that the more immediate, inciting causes may be more closely related. Certainly the limbs, and more especially their distal segments, show a marked susceptibility to unfavorable influences, and in this respect they are in a class with the midline of the back. The reasons for their exceptional vulnerability, however, are not as apparent.

The recognition of predisposing and exciting factors in maldevelopment is tantamount to saying that normal fertilized ova or normal embryos may give rise to malformations. This is undoubtedly true, and Mall was particularly insistent upon it, but in his writings the emphasis is on the exciting or contributing causes rather than upon the deeper predisposing influences. Streeter is much more specific in his reference to eggs of different quality, of varying capacity or potentiality for development, not only as a whole, but in their various derivatives and at different stages of development. Eggs are no more alike or equal than are the individuals from which they were obtained, or the future forms into

which they might have grown. This perfectly natural and normal variability in eggs or embryos expresses itself, in one form, as a varying susceptibility, or resistance to unfavorable influences, and also in the specific type of reaction which such influences may bring out. In the present series of cases we have been dealing with a natural vulnerability of a certain part of the body. It is altogether probable, however, that this vulnerability is not the same in all cases — it may vary in degree or in location, as well as in the disposition of the tissues to react in one way or another. This variability and vulnerability is essentially germinal in character, and for that reason the hereditary possibilities cannot be overlooked.

As pointed out by Streeter, the quality of the egg, and its inherent germinal integrity, determine very largely whether it will succumb early in life, eke out a more or less precarious and misshapen existence, or continue in health and vigor to old age. Even under the most favorable conditions maldevelopment may occur. The more environmental conditions deviate from the normal, the more severe will be the tax upon those factors which should ensure proper development, and the easier it will be for normally latent influences to make themselves felt.

Although the exact role of environmental disturbances in the etiology of maldevelopment is not always clear, such disturbances are especially frequent and conspicuous in young monsters and pathological embryos. Among others, Mall and Meyer have written extensively on the alterations in the embryonic membranes which are so frequent and characteristic, and which undoubtedly play an important part in disrupting or even terminating normal development. As we have nothing out of the ordinary to contribute here, we shall confine ourselves to a brief survey of some of the more general features presented by our material.

There are many ways in which this material is typical of maldevelopment in the human being. Most of the specimens are from the second month. In the cases where the menstrual age is known, it varies enormously, from five and a half weeks to six months, the average being eleven weeks. As a rule the membranes are not normal; the vesicles are often too large, the villi very frequently show hydatid changes in varying degree, or they may be few and small and fibrous. In many cases there is no evidence of any real vascular connection between the embryo and chorion, while exten-

sive subchorionic hemorrhages are very common. The obvious circulatory disturbances may be responsible for the presence of hydramnios, early fusion of amion and chorion, changes in the character of the magma and in the composition of the fluid within the vesicle. In addition to the typical midline defects, many of the embryos show other localized anomalies, while in the majority of them the general condition of the embryo has been considerably altered. The internal disorganization and general disruption, which is so often encountered, is in no sense a teratological condition, but rather a pathological one, although the underlying factors may be much the same. Many of the peculiar skin conditions described above are pathological rather than teratological, if one chooses to draw a line between them, for the inherent vulnerability of certain tissues is quite as much a problem for the pathologist as it is for the teratologist. The various anomalous conditions which are exhibited by the superficial tissues are of very great interest. Two points only need be mentioned here. In the first place, the relatively great expanse of surface exposed to the amniotic fluid would seem to provide some measure of sustenance even after the embryonic blood circulation had ceased entirely. This would apply only to the outermost cells of the body, which might, on this account, be able to prolong their life after the death of all of the deeper structures. In other words, the skin or covering cells of the embryo might, under some circumstances, be the last to die off. The other point is that the amniotic fluid might conceivably act as an irritant to the superficial cells. There seems to be evidence that its character and composition may be altered and in some of our cases one gets the impression that the epithelium has not been living and differentiating under normal external conditions. This hypothetical irritation may, of course, be very mild, behaving more like a stimulant than anything else.

Doubtless the fact that the circulation is so often impaired explains why hemorrhages are relatively infrequent. They occur only in the better preserved, more normal specimens, and here mainly in the region of the head. We have found no indications of hemorrhage or bleb formation in the extremities at all comparable with those described by Bagg [6] in mice. It may very well be that hemorrhage is less frequent on the back than in the hands or feet, on account of the earlier and greater vascularity of the latter. It is also possible

that the relatively poor blood supply of the structures dorsal to the nervous system, especially early, may stand in some relation, or contribute something to the frequency of defects in this region.

It may be objected that some of the conditions described in our embryos are essentially postmortem changes and that it is not possible to attach any particular significance to them. There may be some small justification for this criticism, since, in the very nature of the case, we are dealing, for the most part, with material which is neither wholly normal nor perfectly healthy. In many of our cases, indeed in the most interesting and suggestive ones, there can hardly be any question of postmortem alterations and the picture is anything but that of cell death and a cessation of activity. In some instances there is evidence of hyperactivity, rather than anything else, and indeed some dorsal patches described seem to have retained their vitality longer than any other parts of the embryo. Although we have excluded, as far as possible, all cases which seemed to show only maceration or other moribund changes, it is quite possible that these influences may have contributed something to the general character of the picture in some instances. The variety of conditions observed, and the fact that they occur typically and almost exclusively in the dorsal midline, would indicate the importance of internal rather than external factors in their production, and least of all of postmortem influences.

In the first number of this series on the pathology of development (Ingalls,[1]) we have considered in some detail the general underlying biological and genetic principles out of which flow, naturally and inevitably, certain developmental risks. These risks, or the opportunities for various developmental derangements, are especially in evidence in the structures dorsal to, and including, the central nervous system. It would appear also that these parts of the body are especially sensitive and susceptible in *man*, and this instability, in a sense this relative vulnerability, may express itself in an almost endless variety of ways. The factors at work here are internal, inherent in the nature of the organism, essentially hereditary in character, although altered or disturbed external, environmental conditions may be necessary for, or at least conducive to, abnormal results.

In the present communication we have been concerned especially with some of the milder types of maldevelopment of the back and

head, as exhibited in human embryos of the first two months. They are of interest particularly because these cases may very well represent the earliest stages of some of the anomalous conditions which are encountered in this region at term or even at any time of life. In many of our cases the dorsal defects are very slight and could have contributed little or nothing toward the interruption of the pregnancy, which, but for *other* reasons, might have continued to term. What would have been the final outcome in these cases is, of course, very problematical. Apparently there is, in most instances, either early death of the embryo or fetus from other causes, or a complete healing and restitution of the parts affected. It is quite possible, however, that the damage may be so slight that it is entirely overlooked or its real significance may not be recognized. As noted previously, anomalous or defective conditions of the dorsal midline, varying greatly in degree and character, but not sufficiently severe to compromise further growth and development, are by no means uncommon during the early weeks of intra-uterine life. While many, perhaps most of these cases, fail to go to term, and among those which do reach maturity there may be, in some instances, a more or less complete or adequate *restitutio ad integram*, there remain a certain number in which the initial damage has been sufficiently severe, or of such a character as to render repair or suitable compensation difficult if not impossible. These are the cases which assume a definite clinical, often surgical importance. They are characterized not by any uniformity in the pathology, or in the structural features exhibited by the conditions in question, but rather by their predilection for the dorsal midline, perhaps more particularly the region of the head and neck.

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DESCRIPTION OF PLATES

PLATE 99

FIG. 1. Embryo No. 83. Greatest length about 7 mm. Open neural tube in sacral region. Facial features distorted, heart exposed.

FIG. 2. Embryo No. 46. Greatest length 14.5 mm. The entire body is very much malformed. Almost the whole of the dorsum is markedly altered or defective. The deep triangular cavity is due to the postmortem loss of tissue.

FIG. 3. Embryo No. 129. Greatest length 12 mm., dorsal view. Irregular, roughly circular discolored area in the anterior part of the back. Entire body badly stunted and deformed.

FIG. 4. Embryo No. 161. Greatest length about 12 mm. Symmetrically located, transversely elongated area over the anterior part of the rhombencephalon. Embryo in very poor condition.

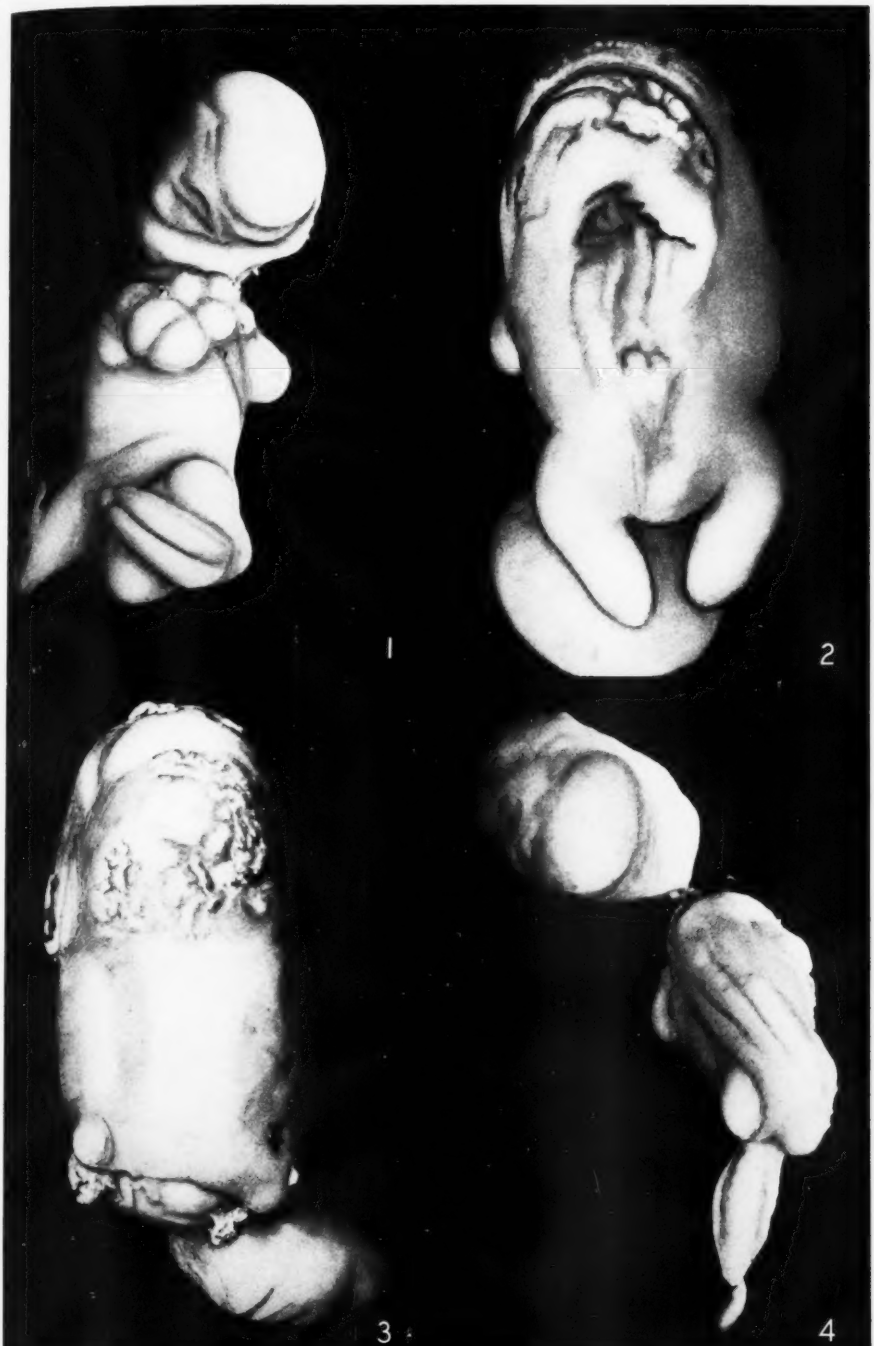
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Plate 100

Fig. 5. Embryo No. 210. Greatest length 15 mm. Large thin-walled bleb in the midline over the rhombencephalon. Much deformity in the body generally.

Fig. 6. Embryo No. 597 B. The smaller of a pair of binoval twins, greatest length 32.5 mm. Very large, rather thick-walled bleb in the midline of head, just behind vertex. Extensive desquamation, malformed hands and cord.

Fig. 7. Embryo No. 407. Greatest length 7 mm. Small bleb-like elevation of epithelium in the midline of the back. Head small and malformed.

Fig. 8. Embryo No. 442. Greatest length 26 mm. Dark patch in lower dorsal region; on the right and below can be seen the borders of the larger area. Laterally the epidermal thickenings are very conspicuous. There is also a very definite transverse band across the forehead.

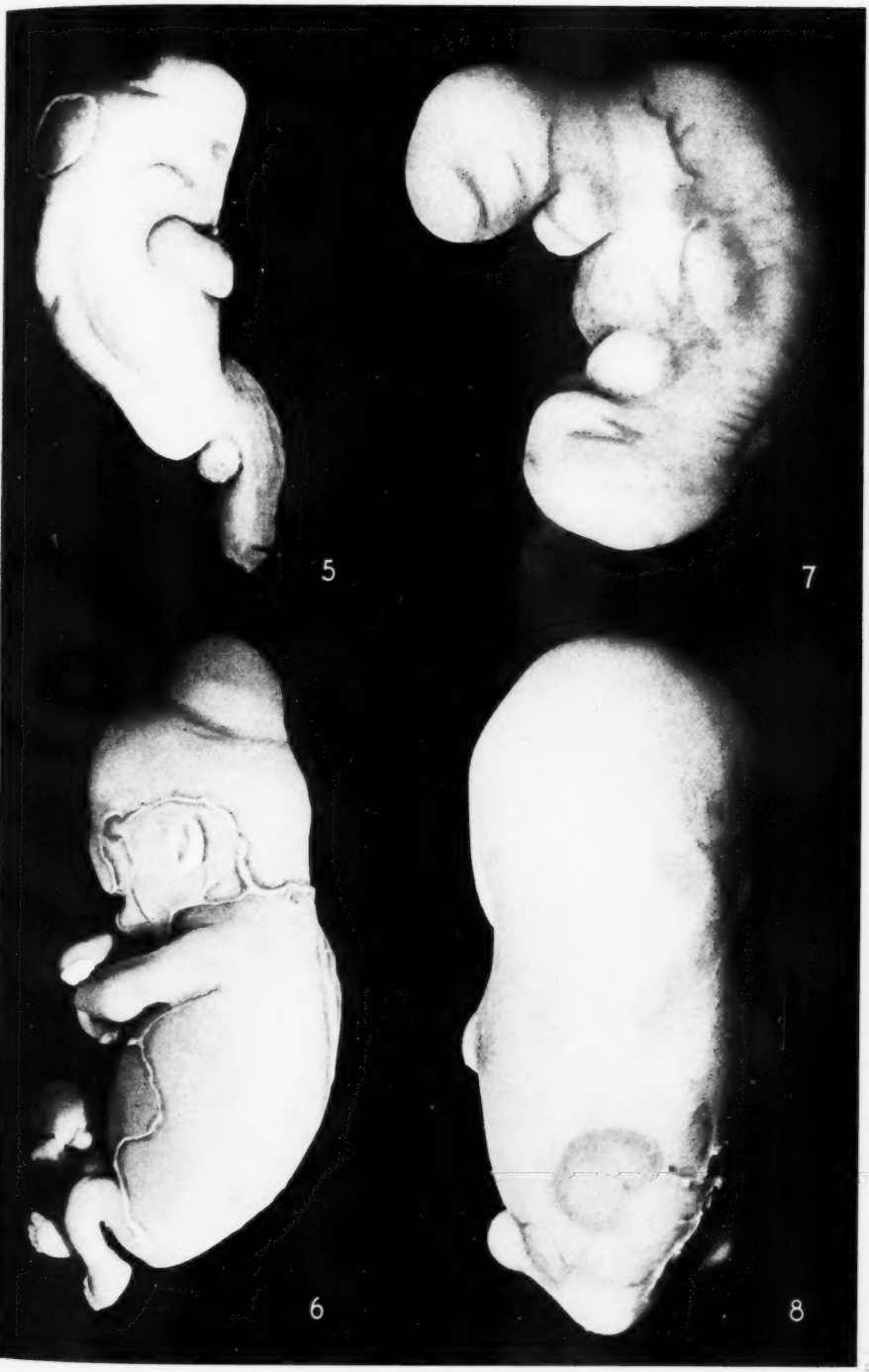
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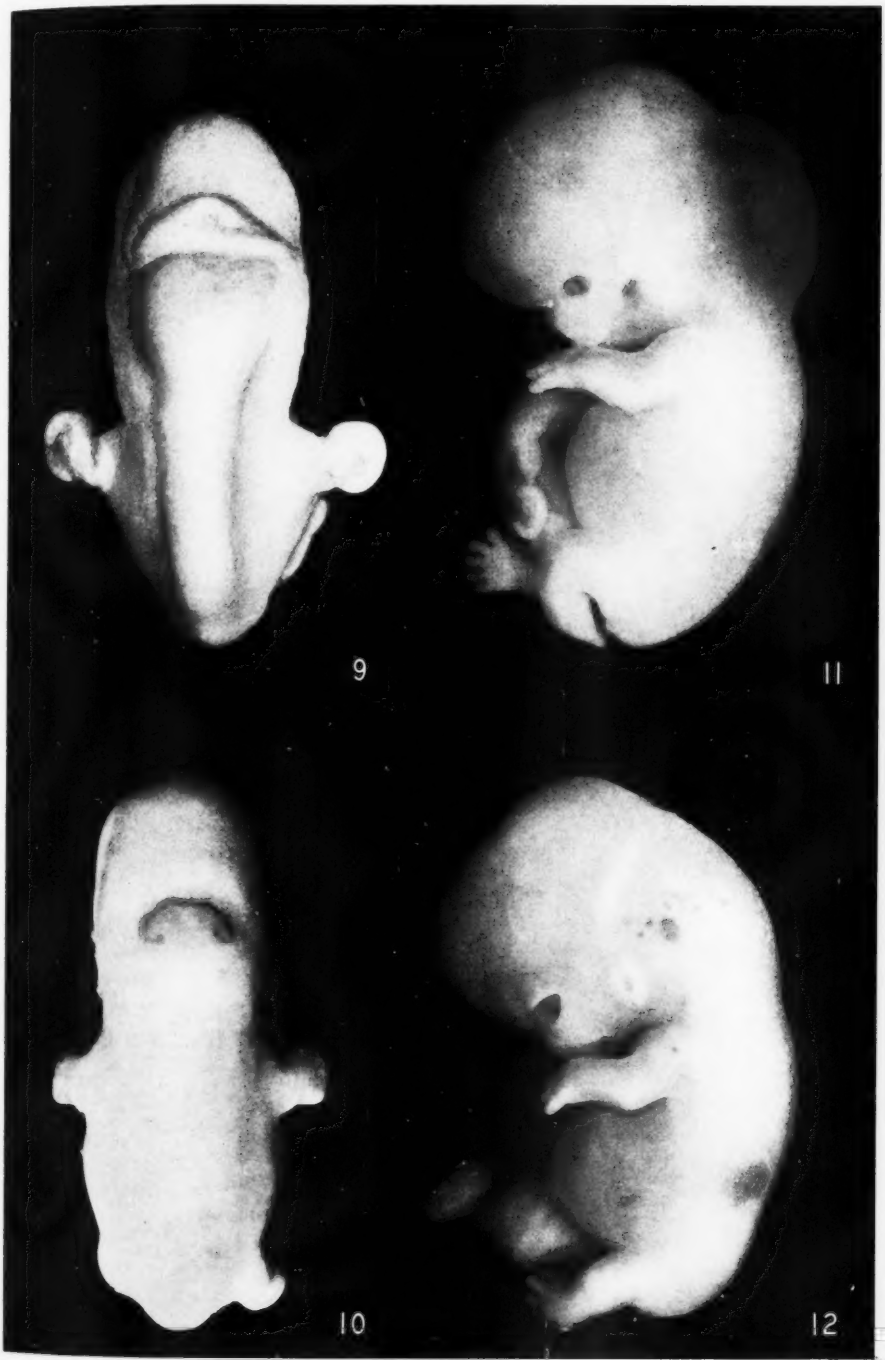
Plate 101

Fig. 9. Embryo No. 652. Greatest length 15 mm. Dorsal view of anterior end of embryo to show the transverse discolored band just behind the midbrain.

Fig. 10. Embryo No. 167. Greatest length 18.5 mm. Very conspicuous, sharply defined, discolored area over the rhombencephalon. There is a similar smaller patch over the vertex and two paired spots on the upper part of the forehead.

Fig. 11. Embryo No. 671. Greatest length 25 mm. Enormous blood-stained bleb on back of head and neck. In the lower thoracic region there is a much smaller one. Small ecchymoses on face and arm.

Fig. 12. Embryo No. 611. Greatest length 23 mm. Minute, translucent bleb over the cerebellum. Ecchymoses on head, shoulder and trunk.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STUDIES ON THE NATURE OF THE NEGRI BODY *

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In spite of the fact that nearly thirty years have elapsed since Negri [1] described the cytoplasmic inclusions associated with rabies, no agreement has been reached concerning their probable nature. Many investigators have worked on this problem and three distinct views are current at the present time.

1. Negri believed that they were protozoan parasites, a contention repeatedly supported by Williams and Lowden,[2] and again by Calkins.[3] More recently evidence in favor of this view has been brought forward by Levaditi and his associates [4] who have, on the basis of their interpretation of the morphological and biological properties and evolutionary cycle of the Negri bodies, proposed the name "Glugea lyssae" for them. Manouélian and Viala [5] have also subscribed to the hypothesis that they are organismal in nature and have considered them to be an aggregation of individual parasites.

2. Other investigators, among whom are Acton and Harvey,[6] Goodpasture,[7] and Cowdry,[8] believe that the Negri bodies arise from injury to certain constituents of the nerve cell brought about by the virus. No agreement has yet been reached as to the actual constituents involved. The neurofibrils and mitochondria (Goodpasture) and the nucleolus (Acton and Harvey) have each been cited as participating in their formation. It has been suggested by Cowdry that a more probable origin is from the Nissl substance.

3. A compromise hypothesis proposed by Prowazek,[9] concerning the Negri bodies and some of the other inclusions in virus diseases, has recently been upheld by Lipschütz [10] and received skeptically by others. According to this hypothesis the inclusions consist of minute elementary corpuscular organisms enclosed in a mantle of substance produced by the cell in response to their presence, that is, they are chlamydozoa, or mantle animals.

* Received for publication March 10, 1932.

A review of the literature upon this subject indicated to us the opportunity of obtaining much more definite evidence by performing a series of investigations using specialized, improved and more recent techniques such as methods to reveal the mitochondria, Nissl substance and neurofibrils; an improved Millon's reagent which we owe to Prof. R. R. Bensley, the Feulgen reaction for thymonucleic acid as specified by Cowdry [11] and the technique of microincineration devised by Policard [12] and improved by Scott.[13]

The Negri bodies were studied (*a*) in the living state under dark-field illumination, (*b*) by vital staining, (*c*) by fixed and stained preparations using selective staining methods and (*d*) by microincineration. The technique and results of each method will be recorded and the significance of these results will then be discussed.

(*a*) *In the Living, Unstained State:*

Observations were made on the Negri bodies in the living condition by dissecting out, in physiological saline solution, a small piece of brain tissue from the region of Ammon's horn in a monkey experimentally infected with rabies. The material was then mounted in physiological saline on a clean slide and the coverglass ringed with vaseline. In this manner it was possible to locate large nerve cells containing inclusion bodies.

By ordinary illumination the Negri bodies appear as definite, almost homogeneous masses, in contrast to the very heterogeneous, granular cytoplasm of the cells containing them. The structure of these bodies is ill-defined, except for the presence of a few, rather dense, light yellow granules (chromatoid granules).

Dark-field illumination, using a cardioid condenser, reveals the Negri body as a clear non-refractile body containing granules corresponding in location to the chromatoid granules. No evidence of a vacuolar structure about each granule or of a membrane separating the Negri body from the cytoplasm is visible. Neither are the granules seen to change their location or exhibit movement.

These observations were more in favor of the theory that the inclusions were reactionary products of the cell constituents, rather than that they were protozoan or organismal in nature.

(*b*) *In Vitally Stained Preparations:*

The application of vital stains, sensitive to oxidation and reduction, to still living nerve cells containing Negri bodies had not previously been attempted, so it was decided to try the effect of

brilliant cresyl blue, Janus green B and Janus red B upon the fresh inclusion bodies, note the staining reaction of their various component parts and possibly gain some information regarding their probable nature.

For this purpose a 1 per cent solution of the dye in 95 per cent alcohol was filmed over a clean slide and allowed to dry. A small piece of Ammon's horn from a rabid monkey was mounted on the slide and spread by means of a platinum loop. A coverslip was then placed over the preparation and ringed with vaseline. As soon as the Negri bodies were located the degree of staining was examined and further changes, like fading and reduction of the dye, were recorded.

Brilliant Cresyl Blue (N. A. C.): With this stain the Negri body as a whole stains a Bremen blue color,* the "chromatoid granules" a Paris blue and the central mass a soft bluish violet. About each of the denser staining components is a less intensely colored region of hyaline material. This imparts to the Negri body a vacuolar appearance about each chromatoid granule, as well as a similarly stained but larger vacuole about the central mass.

In moist preparations the staining was transient, lasting fifteen to thirty minutes; the Bremen blue background of the Negri body was the first to fade and was followed by fading of the granules and central mass.

Fixed preparations which had been air-dried and rapidly dehydrated in absolute alcohol, cleared in xylol, and mounted in balsam, retained the typical staining of the Negri body for several months. Exposure to a carbon arc or other intense sources of light may cause noticeable fading of the staining in from one-half to one hour.

Janus Green B (Grubler): By the use of this dye, employed in the same way as the previous one, it was thought that something characteristic of the Negri body might be found. This dye is capable of being reduced to a pinkish color and then a leucobase, as described by Cowdry.[14] On examination of material treated in this manner bodies were seen in which green granules, corresponding to the chromatoid granules in location and appearance, were embedded in a faint homogeneous material, the latter being the hyaline-like substance of the Negri body. That there is an exchange of substance

* The colors were determined by comparison with Ridgway's standard color scale. (Color Standards and Nomenclature, Robert Ridgway; Washington, D. C., 1912.)

between the Negri body and its cytoplasmic environment is suggested by the reduction of Janus green B by its hyaline matrix which encloses the non-reducing granules.

Janus red B is reduced to a yellow color by the matrix while the granules remain a deep red.

A comparison of the staining reactions with the two Janus dyes and brilliant cresyl blue denotes a less permanent staining on the part of the former dyes with fewer differences in range of color. In other words, the chemical nature of the brilliant cresyl blue permits of a diversity of reaction, the result being that such a dye is of value in locating Negri bodies in brain material from animals suspected of rabic symptoms. The disappearance by fading in moist preparations with the two Janus dyes was essentially similar, the staining of the chromatoid granules being the last to fade.

In addition to yielding information concerning the differential rate of reduction within the Negri body, coloration with Janus green B showed the mitochondria with great distinctness. It is true that the chromatoid granules stained a green of the same intensity as the mitochondria, but after the fading of the mitochondria, the chromatoid granules were found to persist for several hours under proper manipulation.

It is of interest to note the similarity in vital staining between this inclusion and that of vaccinia as described by Cowdry, who came to the conclusion that there was no indication of the presence of independent microörganisms within the latter. The concentration of the Janus dyes used was so high that they stained other elements as well as mitochondria. There was obviously a difference in the reaction to the stain between the mitochondria and the chromatoid granules which did not suggest a common origin as contended by Goodpasture.

(*c*) *In Fixed and Stained Preparations:*

For the purpose of studying the possible part played by the various nerve cell constituents in the formation of the Negri body, materials, in which either fixed or street viruses were used as the infecting agent, were fixed in the following ways: Regaud's formalin-bichromate and Bensley's acetic-osmic-bichromate mixtures for mitochondria, the Hirschler and da Fano techniques for Golgi apparatus, Bethe's method and Cajal's silver pyridine method for

neurofibrils, alcohol fixation for the Nissl substance and Zenker's acetic and formol mixtures for general topographic details.

Preparations stained with aniline acid fuchsin and methyl green showed that the mitochondria, in the case of a monkey infected with street virus, had undergone marked degeneration in some cells. The same was found to be true in cells affected by the fixed virus. Marked changes sometimes occurred in the shape of the mitochondria in the area of the cytoplasm nearest the inclusion body, but possible transition forms between mitochondria and Negri bodies were not observed. Neurofibrils, when examined in preparations treated by the methods mentioned above, were usually noticeably altered, showing fragmentation, thickening and dissolution.

The Golgi apparatus, also, was considerably fragmented in a few of the cells containing inclusions. Examinations of sections prepared for the demonstration of Nissl substance by staining with toluidin blue showed, in cells containing inclusions, a marked variability in the appearance and amount of this constituent, from a state in which most of it appeared to have gone into solution to a condition in which it was very little altered. In the lesions caused by the "fixed" virus of rabies small Negri bodies were frequently seen in a cell at one pole of the nucleus, usually nearest the axone, lying closely adherent to it and embedded in a nuclear cap of Nissl substance.

It was considered essential to determine whether or not the Negri body contains thymonucleic acid, by applying the Feulgen reaction, because considerable emphasis has been laid upon its chromatin content by supporters of the protozoan hypothesis. If a positive reaction were obtained, two alternatives should be considered, each in support of widely divergent opinions; (*a*) that it is organismal in nature, or (*b*) derived from nuclear constituents present in the normal nerve cell.

The material used in this test was obtained from rabbits and monkeys reacting to inoculation with street virus. Portions of Ammon's horn were fixed in equal parts of absolute alcohol and saturated aqueous corrosive sublimate. Sections 3 to 5 microns thick were cut and mounted and the Feulgen reaction applied to them, as described by Cowdry.

In view of the fact that a negative reaction for the Negri body had been reported by Paul and Schweinburg [15] and that normal nuclei of Ammon's horn give a relatively weak reaction, control material (spleen and liver) was subjected to the same treatment to assure ourselves that the reagents used were suitable. It was found that variable results were obtained. In some Negri bodies the reaction was negative, while in others a faintly positive result was secured corresponding in location to the chromatoid granules within the inclusion.

It was then decided to apply the Millon test, with reagents prepared under the direction of Dr. R. R. Bensley, to sections of brain tissue of rabbits containing Negri bodies. The results were found to agree closely with those secured by the Feulgen reaction, *i. e.*, there were occasional faintly positive reactions on the part of individual granules within the inclusion bodies. It is of interest to note the instances in which a feeble reaction is obtained, indicating the presence of proteins probably somewhat altered or reduced in amount, due to the degenerative changes occurring in the cell.

Macallum's iron reaction, as modified by Nicholson,[16] was applied to material fixed in 95 per cent alcohol. A very faint reaction on the part of the chromatoid granules and central mass indicated the presence in them of masked iron.

The variability of the Feulgen reaction does not agree with the protozoan hypothesis, as presumably this reaction would have been more consistent were this the case; therefore, we interpreted the result of this test and the Millon test as indicating the presence in some inclusions of chromatin of nuclear origin which was not present in others, for example the "lyssa bodies."

The changes which occurred in the various cell constituents did not aid materially in reaching a decision as to which was mainly involved in the formation of the Negri body, if such a phenomenon occurred. However, the earliest forms of Negri bodies were seen to lie in a cap of Nissl substance, which indicated that possibly this observation had some significance in view of the results of the Feulgen test and the masked iron reaction.

(*d*) *In Incinerated Sections:*

The inorganic constituents of the Negri body were investigated by the technique of microincineration. It was hoped that by so doing decisive evidence would be secured bearing upon the question

as to whether it is protozoan in nature, or a product of cellular constituents altered by the action of the virus.

The technique employed was that devised by Policard and modified by Scott. The material used was taken from the region of the hippocampus of a monkey which was experimentally infected with street virus and sacrificed 18 days after inoculation. This material was fixed for 24 hours in a solution containing 9 parts of absolute alcohol to 1 part of formalin, after which it was thoroughly dehydrated in absolute alcohol, cleared in xylol and mounted in paraffin. Sections 4 microns thick were cut and mounted on slides, using liquid petroleum to prevent any absorption of water. The slides were then placed in a quartz oven, the heat of which could be regulated by means of a rheostat, and incinerated for 35 minutes at temperatures gradually increasing from 143° C to 604° C. After cooling they were removed and mounted dry. Alternate sections to those incinerated were stained in the usual manner for controls.

The incinerated sections were examined under high dry and oil immersion lenses, using dark-field illumination. The results obtained by this method proved very satisfactory because examination of the control sections showed that the finest topographic details had not been lost by the incineration. The ashes left from the inorganic and organically bound salts within the nerve cells were clearly demonstrable and the cell membranes, nuclear membranes and nucleoli were very evident (Figs. 1 and 2).

The nature of this ash is to some extent revealed by its color. Calcium, magnesium and aluminium appear as a grayish white deposit, organically bound iron as a faintly yellow ash, while free iron is reddish in color.

The distribution of the ash in sections of normal nerve cells examined showed that the nucleolus left a distinct yellow ash, indicating that iron-containing protein is present in comparatively large amounts in that structure. The nucleus contained mainly a deposit of grayish white ash in which a fair number of yellow particles were apparent; and the cytoplasm a large quantity of grayish white ash, probably mostly calcium, with a much smaller, scarcely visible amount of yellow residue.

Similar areas of a control section showing numerous Negri bodies were compared with those of the incinerated material. In the latter a number of cells were seen to exhibit compact ash deposits occupy-

ing such positions within the cytoplasm as to leave no doubt in our minds that they constituted the residue of incinerated Negri bodies (Figs. 5 and 6).

It was impossible to reproduce in the photographs all the details observed in the sections. It can be seen, however, that there is a decided difference in the ash of the nucleus and cytoplasm of cells containing Negri bodies (Figs. 5 and 6), and those without inclusions or of normal cells (Figs. 3 and 4). Camera lucida drawings were made of incinerated cells containing Negri bodies and similarly treated normal cells which show more clearly the difference in the orientation and amount of ash (Figs. 7 and 8).

In cells containing Negri bodies there appears to be an orientation of the ash around the periphery of the nucleus and the cytoplasmic ash is very much reduced, apart from that of the inclusion. There also appears to be a reduction in the amount of ash in the nucleolus.

In a number of Negri bodies examined there was a variation in the organically bound iron present. Stages from a practically iron-free deposit to others in which the iron particles were scattered throughout the cell body, giving it a distinct yellow appearance, were observed. It is clear, therefore, that the incineration of Negri bodies at high temperatures leaves a grayish white ash consisting mainly of calcium, together with a variable amount of organically bound iron ash.

There was no suggestion of any inorganic residue which might represent a membrane surrounding a protozoan, neither did the internal structure of this fairly compact ash, which resulted from the incineration of the Negri body, suggest an aggregation of individual parasites within the body. This was of interest because Scott and Horning [17] in their work on opalinids found that the nuclei of protozoa contained very little ash; and no almost ash-free structure, representing a possible protozoan nucleus, could be distinguished within the incinerated Negri body. The discovery of yellow "masked" iron within some of the inclusions supports the results obtained with the Feulgen, Millon and Macallum reactions and will be referred to in the discussion.

Discussion

These observations have, we believe, a definite bearing upon the three hypotheses concerning the nature of the Negri body.

1. The hypothesis advanced by many authors that the Negri bodies are individual protozoa, or by others that they are an aggregation of individual parasites does not, in our opinion, appear to be supported by the results obtained in these experiments.

Under dark-field illumination in the living state no evidence of a membrane separating the Negri body from the cytoplasm of the cell was seen, neither did the granules change their location or exhibit movement. The microincineration technique, by which a plasma membrane is rendered visible when present, also supported the previous observation by being negative in this respect. Furthermore, the nucleus of a protozoan (opalina) has been observed by Scott and Horning to contain very little ash, which did not agree with the rather compact ash residue within the Negri body; but of course it does not follow that all protozoan nuclei are characterized by a light ash, so that this observation, though of considerable interest, is of little value as evidence.

The variability in the Macallum, Feulgen and Millon reactions was less in favor of the protozoan contention than of the origin of the inner bodies from extruded basophilic nuclear chromatin and Nissl substance, unless it is assumed that the Negri bodies are dead and dying protozoa.

Again, the observations of other authors, such as Acton and Harvey, are not in agreement with this theory. They state that the extreme variations in size and appearance of these inclusions in the various experimental animals suggest their formation from cytoplasmic material, rather than the interpretation that they are protozoan in nature. It is true that no protozoan has ever been reported which at times is invisible and which exhibits such profound differences in size and appearance when parasitic in different species of animals. The variation between the "lyssa body," as described by Goodpasture, and the typical Negri body would also suggest the same conclusion. Neither does the experimental evidence, which we do not intend to discuss in this paper, lend strength to the idea that the Negri bodies are protozoan.

2. The concept that the Negri bodies are formed from constituents present in the normal nerve cell is more in agreement with our findings. Evidence has already been brought forward that the neurofibrils and mitochondria (Goodpasture), and the nucleolus (Acton and Harvey), take part in their development. It has also been suggested by Cowdry that the Nissl substance may contribute to their formation.

With regard to the contention that the mitochondria and neurofibrils give rise to the formation of this inclusion body, it does not seem to us that the evidence is very conclusive, especially regarding the former. The material we used for microincineration had previously been fixed in an absolute alcohol mixture in which the mitochondria would in all probability have undergone solution, and corresponding ash-free areas, occupying the position of the inner bodies (chromatoid granules), to which they are alleged to give rise, would have been present in the incinerated Negri bodies. These were not present. In the stained control sections from material treated in the same manner these inner bodies were clearly demonstrable, which would further indicate that their origin from mitochondria was highly improbable. Moreover, there was the evidence from vital staining which indicated a difference in the chemical nature of these elements, the chromatoid granules retaining their staining for some time after the mitochondrial staining had faded. The former occasionally reacted positively to Macallum's test for masked iron and the Feulgen reaction, whereas the latter never did, and again, the chromatoid granules are basophilic while the mitochondria are acidophilic. Evidence is therefore lacking of transition between the mitochondria and the chromatoid granules.

Although the neurofibrils showed marked degeneration we could not distinguish within the Negri body, located in a cell showing these changes, any sign of material suggesting the formation of its hyaline substance from this constituent. However, the evidence of Goodpasture is more suggestive regarding the neurofibrils, and the possibility of their playing some part in the formation of the hyaline substance of the Negri body cannot be disregarded. The difficulty is that the neurofibrils are seldom, if ever, visible in the living nerve cells of mammals, while the Negri bodies always are. There is reason to believe, as Cowdry [18] insists, that methods of impregnation like those we have employed tend to exaggerate their size so

that apparent similarities are to be discounted. Obviously to reach a decision the two must be compared side by side in the same cells by a variety of techniques in addition to the one used by Goodpasture which, like the silver method, reveals the neurofibrils in an unusually robust state. So elusive are the neurofibrils that we know nothing of their chemical composition and consequently cannot speak with assurance regarding their change into other substances.

The Golgi apparatus, although fragmented in many cells containing inclusions, did not appear to have any relation to their origin.

However, the results we obtained from the microincineration technique and masked iron reaction indicated the presence of organically bound iron, and the strikingly similar results from the Feulgen and Millon's reactions indicated the presence of chromatin of nuclear origin. Both types of reactions occurred within the typical Negri body in the position of the inner bodies or chromatoid granules, and were interpreted by us to be due to these bodies having their origin in the basophilic, iron-containing protein of the nucleus which had been extruded into the cytoplasm during the cellular reaction to the virus. The Negri bodies which gave a negative reaction to the above techniques we interpreted, on the basis of Macallum's theory on the conversion of basophilic chromatin into oxyphilic granules, as consisting of material which had lost its basophilic properties and become oxyphilic. Inclusion bodies which are acidophilic include the presumably atypical inclusions which Goodpasture terms "lyssa bodies."

The presence of organically bound iron within the typical Negri bodies led us to review the available literature upon this side of the question. A great deal of work has been done in connection with the iron-holding chromatin of the cytoplasm (Nissl substance) and the chromatin of the nucleus. As early as 1897 Mackenzie [19] and Macallum [20, 21] showed that this so-called chromidial substance was rich in iron, and the former was the first to report the presence of organically bound iron in nerve cells. He further pointed out the relationship between the iron-holding Nissl substance (chromidial substance) and the iron-containing chromatin of the nucleus. In his work upon the nerve cells of rabbits which had been inoculated with rabies he found that as long as basophilic granulations were present in the cells, iron-holding material was also present. He further reported a factor of importance to our interpretation, in so far

that in the cortical motor cells of an animal suffering from rabies there was evidently a conversion of the iron-holding basophilic chromatin into oxyphilic granules containing very little iron, and these oxyphilic granules corresponded to the previous location of the Nissl substance. It is known that the Nissl substance undergoes characteristic changes during fatigue and injury, and Nicholson, in his extremely interesting work upon the changes in iron substance in nerve cells, pointed out that the alteration in the iron-reacting granules of the cytoplasm corresponded with the morphological changes in the Nissl substance in nerve cells following injury. It is also pointed out that a regeneration of iron-holding chromatin occurs apparently from the nucleus and in all probability the Nissl substance is eventually replaced around the nucleus if complete recovery of the cell occurs. We maintain that this evidence is in support of our observations on the origin of the "lyssa bodies" and on the variation in amount of Nissl substance in nerve cells containing either "lyssa bodies" or Negri bodies.

According to Scott,[22] apart from the Nissl substance, the covering of the nucleolus and the nuclear chromatin also contains iron. He found that in pig embryos, 7 mm. to 18 mm., the iron-holding chromatin is confined to the nucleus, but that during further development the basophilic iron-holding chromatin passes into the cytoplasm but the oxyphilic does not. Other authors believe that there is a passage of the iron-containing protein of the nucleus through the nuclear membrane into the cytoplasm during chromatolysis; this phenomenon might be expected to occur as an endeavor on the part of the cell to preserve a nucleocytoplasmic ratio compatible with life.

It is very significant that in the incinerated sections of cells containing Negri bodies there is an orientation of the nuclear ash around the periphery of the nucleus, and within the Negri body in the same cell it is easy to detect the yellowish ash, which represents the organically bound iron. Likewise, when present, the chromatoid granules of the Negri body invariably show a basophilic staining reaction similar to the basophilic nuclear chromatin. Again the Feulgen and Millon techniques gave a faintly positive reaction corresponding in location to those basophilic granules within the inclusion.

If we accept the observations of Scott, Nicholson and other au-

thors, it seems to us that there is now sufficient evidence to account for the origin of the Negri body with its many variations in structure.

It is our belief that owing to the action of the virus (enzymatic, catalytic or otherwise) there is a reaction bringing about chromatolysis with its accompanying alteration of the Nissl substance and chromidiosis. The basophilic iron-containing protein of the nucleus is extruded into the cytoplasm, probably in an endeavor to support the proper nucleocytoplasmic ratio; this in turn is also gradually converted, during the cellular reaction, into the oxyphilic, apparently homogeneous material of the matrix of the Negri body; the remaining unaltered portion or portions forming the basophilic iron-containing inner bodies.

The "lyssa bodies" may, in our opinion, either result from the complete conversion of all this basophilic chromatin into oxyphilic material, or by an aggregation of the particles of altered Nissl substance which during chromatolysis had lost its iron-containing properties.

The remarkable similarity of the variations in the results obtained with the microincineration, masked iron, Feulgen and Millon techniques appeared to us to bear a striking resemblance to those which would be produced as a result of the formation of the various types of Negri bodies, including the "lyssa bodies," from the basophilic nuclear chromatin and Nissl substance due to the action of the virus of rabies. Obviously, however, it cannot be said that either Negri bodies or lyssa bodies are formed wholly from basophilic material. It is impossible to exclude the ground substance of the cytoplasm, containing the Nissl substance, which does not color with basic dyes, for the material of the Nissl bodies is probably present during life in solution in it (Cowdry [23]).

3. The compromise hypothesis that the Negri bodies consist of minute elementary corpuscular organisms enclosed in a mantle of substance produced by the cell in response to their action is, owing to its very nature, difficult of proof or disproof. If the elementary corpuscles were microscopically visible it would not be so difficult. The trouble is that we and others have consistently failed to find them. They are by definition different from the "chromatoid granules" which are seldom very numerous and which vary in size, sometimes being comparatively large. If such elementary organisms

do exist one would expect them to be at least slightly basophilic, for the tiniest organisms, the Rickettsia, are basophilic. Yet in many Negri bodies no trace of basophilia can be distinguished. For reasons such as these the chlamydozoal hypothesis can only be dismissed as not proved.

Conclusions

1. The cytological evidence presented, with that of other authors, together with the experimental evidence, is not compatible with the protozoan or organismal theories concerning the nature of the Negri bodies.

2. The contention that they arise from constituents already present in the nerve cell as a result of the action of the virus is in agreement with our findings, but we consider the evidence for the participation of the mitochondria, neurofibrils and nucleolus as inconclusive.

3. Both the Negri bodies and the smaller atypical lyssa bodies are probably formed by alterations in the basophilic Nissl substance, the fundamental ground substance of the cell, and by addition of variable amounts of basophilic material of nuclear origin. There is no evidence that organisms on the borderline of microscopic visibility are cloaked with these cellular components in accordance with the chlamydozoal hypothesis.

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DESCRIPTION OF PLATES

PLATE 102

FIGS. 1 and 2. Showing the comparison between a stained control section and an incinerated section taken from the same portion of brain tissue. The fact that the majority of the histological detail is preserved after incineration can be seen in spite of the loss of detail due to refraction of the light upon the mineral salts during photography.

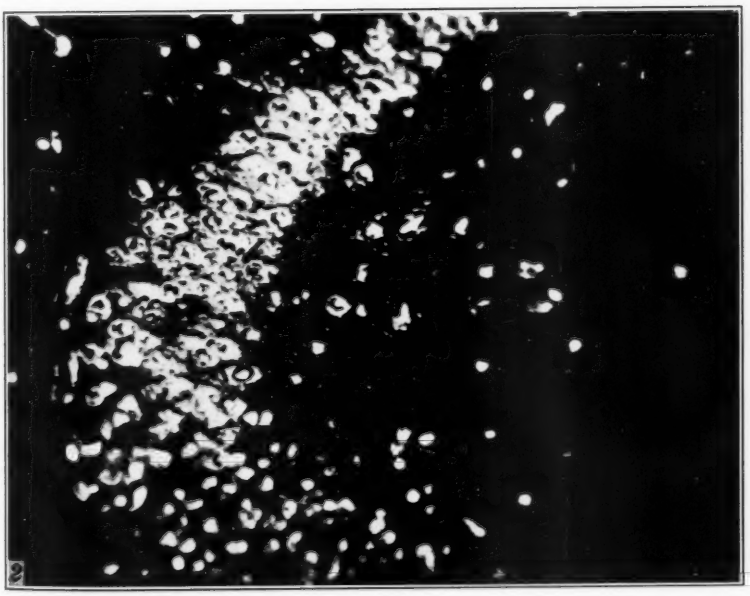
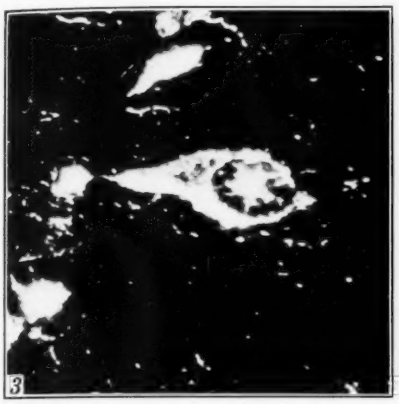


Plate 103

Figs. 3 and 4. Normal incinerated nerve cells showing the amount of mineral ash present in the nucleus and cytoplasm.

Figs. 5 and 6. Cells showing the rather compact ash deposit, lying close to the nucleus, which represents the mineral salt content of the Negri body.

Figs. 7 and 8. Camera lucida drawings indicating the difference in the inorganic ash after incineration of normal nerve cells (7), and cells containing Negri bodies (8). Note the orientation of the ash of the nucleus around its periphery in the latter and also the loss of the cytoplasmic mineral salts, apart from those in the Negri body.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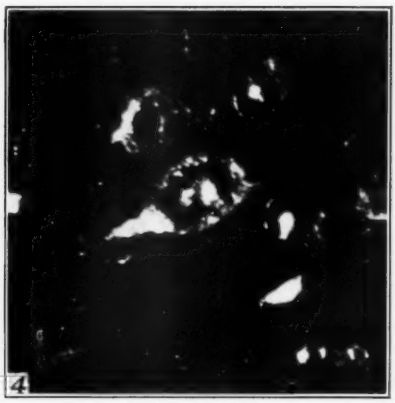
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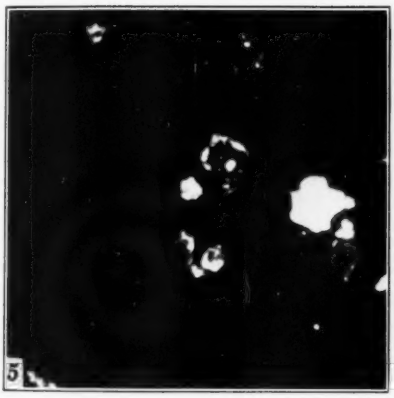
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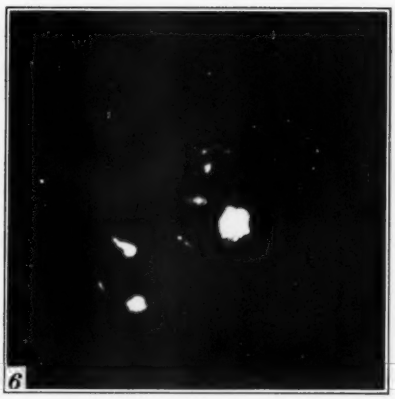
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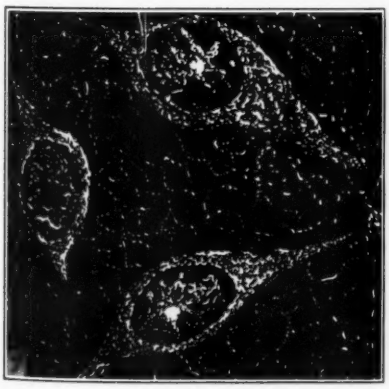

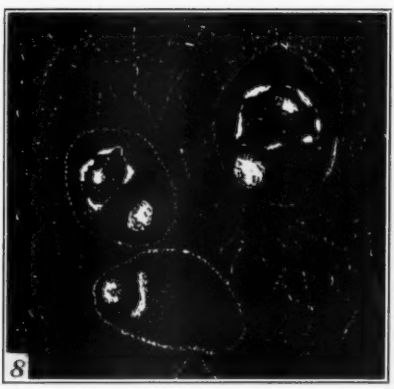
8

THE CIRCULATION IN THE PANCREATIC LOBULE AFTER PARTIAL VENOUS OBSTRUCTION *

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The object of the studies here reported was an analysis of the circulatory changes in the pancreatic lobule during venous obstruction. Little attention had been directed to the changes in the pancreas in chronic passive congestion until 1925, when VonGlahn and Chobot[1] described in detail for the first time the changes in the pancreas in cases of heart failure. Among the distinctive features observed were areas of capillary congestion at the periphery of the primary lobules, with no congestion of the vessels of the islands of Langerhans. The observations emphasized the importance of a careful study of the capillary patterns in these lobules.

Before 1925, however, DeWitt[2] had made a comprehensive study of the anatomy and physiology of the pancreas. Relative to the circulation she believed that the islands of Langerhans were supplied by venous channels arising from nearby veins. She found that there was stasis of blood in the island capillaries in chronic passive congestion and cites this in support of the venous origin of these vessels. Since this work, few investigations concerning the circulation in the pancreatic lobules have appeared.

Beck and Berg[3] made an anatomical study of the circulatory pattern in the islands of Langerhans and found that the islands were located near the larger vessels of the lobules. They described short direct arterioles supplying the island capillaries, a free anastomosis between these capillaries and the interacinar capillaries, and short direct efferent venules draining the island network. Thus they were presented as units lying near the central vessels of the lobule, having a distinct and separate blood supply independent of the capillaries of the remainder of the lobule, except for the free anastomosis occurring between the insular network and the interalveolar rete. It

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was also emphasized that if an island were large, more than one arteriole supplied its network — as many as three arterioles to some of the larger ones. These short direct arterioles to the islands were contrasted with the longer ones supplying the outlying acinar tissues of the lobule.

It was explained that this arrangement favored the circulation of a large volume of blood through the islands. Since the arterial pressure diminishes directly with the length of the vessel, it is higher at the end of a short arteriole than at the end of a longer one. Therefore, the short direct arterioles to the islands afford a higher pressure for the column of blood entering the capillary network than the longer arterioles supplying the outlying acinar tissues. On the venous side the short direct venules of the islands ensure a low venous pressure with a minimal resistance to the egress of blood. As a result of these observations it could be stated that in chronic passive congestion the circulation through the islands is maintained because the pressure in the short arterioles is sufficient to overcome the increased pressure in the venous system. On the other hand, there is stasis in the peripheral parts of the lobules because the pressure in the longer vessels is not sufficient to overcome the venous pressure.

In the following experiments increased venous pressure in the veins of the pancreas of rats was produced by partially obstructing the flow through the inferior vena cava between the base of the heart and the diaphragm. This caused congestion of the portal venous system through the liver, pancreas and intestines. In all, about twenty-five adult white rats were used. One was used as a control without obstruction and in this instance the dye used circulated uniformly with the blood without evidence of emboli occluding arteries or arterioles. The total flow was obstructed in three rats — in the remainder the obstruction was partial.

Technique

The white rats were anesthetized with sodium luminal.* A tracheotomy was done and the cannula of a small pulmotor was inserted and tied in place. The thoracic and abdominal viscera were exposed through a midline incision extending from the symphysis to the manubrium. Two lateral incisions were made, extending from

* 0.13 gm. subcutaneously is usually sufficient to anesthetize an adult rat. The animal is usually adequately anesthetized within thirty minutes.

the subcostal angles and passing up the tenth intercostal space on each side toward the axillae. Such incisions usually avoid large arteries and veins so that hemorrhage is not troublesome. The four flaps formed by the incisions were retracted and held in place by clamps. This gave full exposure of both thoracic and abdominal viscera. Warm saline was used over the viscera to protect them from drying. The diaphragm was cut from its anterior and lateral attachments and the phrenic nerves were severed, thus paralyzing the diaphragm. By these procedures about 2 cm. of the inferior vena cava was exposed between the diaphragm and the heart. A ligature was placed loosely around the aorta just above the heart so that it could be tied immediately after the injection had been completed. By means of a soft copper wire placed around the inferior vena cava varied degrees of partial obstruction in some cases, and complete obstruction in others, were made. With the pulmotor working, the circulation could be kept going for periods sufficient to ensure definite enlargement of the liver and distention of the veins of the viscera. When the congestion was well marked the pericardium was opened and by means of a small hypodermic needle Higgins' commercial India ink was injected slowly into the left ventricle. The injection was continued until the liver became "peppered" with pinpoint black dots. The aorta was then tied and the animal immediately placed in cold 10 per cent formalin. Later the pancreas was dissected out and the lobules were teased apart and studied. Some lobules were sectioned with a razor blade and others were embedded in paraffin, sectioned and stained with hematoxylin and eosin. By far the best specimens were obtained by dehydrating, clearing and mounting whole lobules after they had been teased apart. They were cleared in methyl salicylate.

The Circulation in the Lobule without Obstruction

The capillaries of the lobules in these experiments were uniformly injected and there was no evidence of emboli obstructing the flow. The islands stood out as darker areas located near the larger vascular trunks (Fig. 1). These sections were used as controls.

The Circulation in the Lobule with Partial Obstruction of the Inferior Vena Cava Immediately Proximal to the Diaphragm

The peripheral capillaries were dilated and engorged with red blood corpuscles, while the arteries and arterioles were filled with black dye. The capillaries of the islands of Langerhans were well injected and stood out as black areas of tortuous capillaries (Fig. 2). There were occasionally seen other areas of partial injection, usually located near the islands. The short arterioles to the islands were blackened; the efferent venules were also, but to a lesser degree. In the long arterioles the dye was dense at the proximal end and a short distance along the course. The dye gradually faded before the peripheral capillaries were reached. The venules draining the peripheral capillaries were widened and engorged with red blood corpuscles and contained no dye. The arteries and arterioles in the lobules of the many pancreases examined were similar, but varied in dye distribution according to the amount of obstruction of the inferior vena cava. In every case in which the degree of obstruction was just short of being complete (the degree estimated at the time when the copper wire was placed around the inferior vena cava), there was an absence of dye in the periphery. In those cases in which the degree of obstruction was estimated as about one-half, a small amount of dye flowed into the peripheral capillaries. However, the capillaries of the islands contained dye in all degrees of obstruction with the single exception of complete obstruction.

The Circulation in the Lobule Following Complete Obstruction of the Inferior Vena Cava Immediately Proximal to the Diaphragm

In these cases the intralobular arteries contained the black dye. The arterioles, both short and long, and the capillaries of both the islands and acinar tissue were free from it. The venules were markedly dilated and engorged with blood, as were the capillaries. The peripheral capillaries were more markedly dilated than those in the pancreatic islands (Fig. 3).

Physical Model

The findings in these experiments were more easily interpreted when the pressure relationships were more accurately and critically examined in a physical model so constructed as to conform closely to the circulatory pattern in the pancreatic lobule.

Such an apparatus (Fig. 4) may be constructed with the use of a reservoir, a U-tube equipped with piezometer tubes, and suitable connections for incorporating a connecting-tube and constrictions. Each limb of the U-tube may conveniently be about 45 inches long with piezometer tubes erected at 15 inch intervals. The bend may also be 15 inches long. One limb (proximal) is attached to the lower end of the reservoir. The reservoir is of a type which maintains a constant level while the fluid is flowing through the system. In comparing the apparatus to the vascular system in the pancreatic lobule, the reservoir is the source of steady pressure and represents the intralobular artery. The proximal limb from the reservoir to the bend represents the small arterial branch arising from the intralobular artery which will carry blood to the peripheral parts of the lobule. The bend of the U-tube represents the peripheral capillaries, and the distal limb may represent the small vein which brings blood from the periphery back to the middle zone of the lobule. The circulation through the islands is represented by a connecting-tube (15 inches long) inserted into the system to join the proximal and distal limbs a short distance (about 15 inches) from the reservoir. The short arterial branch from the intralobular artery is represented by the segment of the proximal limb of the U-tube existing between the reservoir and the point of attachment of the connecting-tube; the insular capillaries are represented by the connecting-tube, and the short efferent veins are represented by the segment of the distal limb existing between the outlet of the system and the point of attachment of the connecting-tube.

The piezometer tubes are so placed as to indicate the pressure in the critical points of the system. Along the proximal limb the piezometer tube (P_1) is near the reservoir and adjacent to the connecting-tube, P_2 is between the connecting-tube and the bend, and P_3 is nearer the bend. Along the distal limb P_4 is near the bend, P_5 is between the bend and the point of communication made by the connecting-tube, and P_6 is adjacent to the connecting-tube.

The height of the fluid in the piezometer tubes, the speed of flow throughout the system, and how these are affected by varying degrees of obstruction at the outlet are more easily understood if the simplest possible condition exists. To begin with, therefore, the connecting-tube is omitted and the conditions examined in the simple U-tube. To observe the speed of flow in the system a dye may be injected through a rubber connection with a needle and syringe at any desired time. The rate at which the dye moves along indicates the speed of flow with accuracy sufficient for this experiment.

With a set level in the reservoir, and while the fluid is flowing freely through the system, the fluid level in P_1 is highest, and in P_6 it is the lowest. Between these points is a gradual uniform change in pressure, which, if charted, will form a straight line between the highest pressure and the lowest pressure. Thus is formed a uniform gradient of diminishing pressure along the length of the U-tube (see curve "a" on the chart). The pressure at any other point can be computed by hydraulic formulae. By partially occluding the outlet while the fluid is flowing, a change in pressure occurs at once throughout the system and is seen in the rising levels in the piezometer tubes. It is observed that the greatest change in pressure will be in the piezometer tube nearest the obstruction, and the least in the one nearest the reservoir. Between these points the change is intermediate. If the heights of the new levels (see curve "b" on the chart) are plotted on the previous chart the change is apparent. At once it is seen that a uniform diminishing gradient of pressure, caused by the obstruction, is formed between tubes P_6 and P_1 against the flow. The new gradient is formed at the expense of the gradient of pressure arising from the reservoir, and the speed of flow is lowered. As the obstruction is increased toward completeness the levels approach each other, the gradient becomes less and less, and the speed of flow becomes slower and slower. When the obstruction is complete, the levels are equal to each other and to that in the reservoir; there is no gradient and the flow is nil.

By inserting the connecting-tube to represent the circulation through the islands a slightly different set of conditions is produced. There is noted a slight change in the levels in the piezometer tubes when the flow through the tubes is established. There is a fall of the levels in the tubes of the proximal limb (see curve "x" on the chart). The greatest is in P_1 near the connecting-tube. The levels in the

tubes of the distal limb (P_4, P_5, P_6) are slightly elevated. The greatest change is in tube P_6 which is nearest the connecting-tube. The reason for these changes is readily seen when it is remembered that the connecting-tube allows a flow from the highest pressure of the proximal limb to the lowest pressure of the distal limb; it lies between the extremities of the gradient of pressure in the system. Thus a rapid flow is established, which allows a fall in pressure from the proximal limb and causes a slight rise in pressure in the distal limb as described. There follows a disturbance in the original uniformity

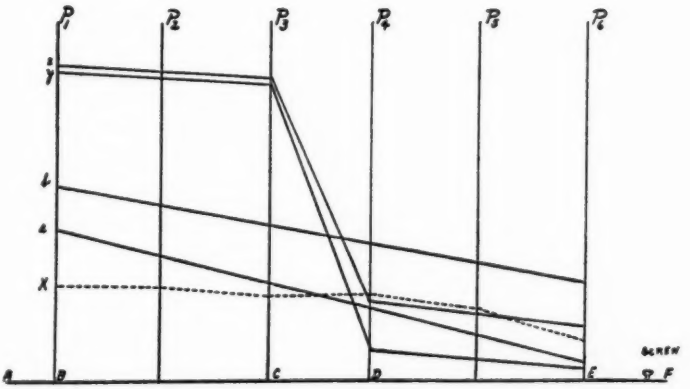

CHART 1

Curve "a" shows the gradual uniform change in pressure between piezometer tubes P_1 and P_6 with only the U-tube and no connections.

Curve "b" shows the uniform increase in pressure between tubes P_1 and P_6, with partial occlusion at outlet in the U-tube with no connections.

Curve "x" represents the pressure after insertion of connecting-tube between the limbs of the U-tube.

Curve "y" represents the pressure in the tubes when the outlet has been partially obstructed and with the connecting-tube inserted.

Curve "z" represents the pressure when constrictions have been made in bend of U-tube and also in the connecting-tube, and with partial obstruction to the outlet.

of the gradient, which tends to approximate the pressure in the tubes P_3 and P_4 at the bend. At the same time there is less pressure to force the fluid along, as much of it is shunted through the connecting-tube. This results in a slowing of the flow through the bend.

If the outlet is again partially occluded while the fluid is flowing

there is again noted a sharper rise in pressure in the system near the obstruction. The rise becomes less and less as the reservoir is approached. The gradients are similar to those following obstruction in the previous experiment, with the exception of the slight alteration caused by the connecting-tube, the result being that there is greater slowing of the flow through the bend. The slowing is progressive as obstruction approaches completeness.

There are many islands, however, whose afferent arteries arise directly from the intralobular arteries instead of from the small arterial branch illustrated in the model. In this case the free anastomosis existing between the capillaries of the islands and those of the adjacent acinar tissue makes a similar relationship of pressure, though the amount of variation may be slightly less. The efferent venules are more commonly found as tributaries of a small vein which drains a part of the interacinar capillaries.

If a resistance like that of the capillaries of the periphery is now put into the system by means of a constriction in the U-tube at its bend, and a similar constriction is placed in the connecting-tube to represent the resistance offered by the insular network of capillaries, the apparatus more nearly represents the situation in the pancreatic lobules. The greater resistance of the interacinar capillaries may be represented by a constriction in the bend of the U-tube, which is narrower than the one in the connecting-tube which represents the slightly wider capillaries in the islands. As a result of these changes in the apparatus there is a marked alteration in the levels in the piezometer tubes. Those in the distal tube (P_6, P_5, P_4) are lowered somewhat, while those in the proximal tubes (P_1, P_2, P_3) are greatly elevated (see curve "y" on the chart). The explanation[4] of these changes is simply the result of an adjustment of the pressures to the resistances placed in the apparatus. The less resistance in the connecting-tube will allow a greater volume of fluid to flow at a faster rate between the extremes of pressure. This will result in a still slower rate in flow through the smaller constriction at the bend, and is explained by the same principles above described when the capillaries of the U-tube and connecting-tube are uniform. If partial obstruction is applied at the outlet as before, there is, as a result, a much more marked slowing of the flow through the bend than was observed in any of the previous conditions. The slowing through the connecting-tube is not so marked. As obstruction approaches com-

pleteness the rate of flow in the bend is so slowed that the movement of the fluid is barely perceptible, while that in the connecting-tube is readily perceived.

Discussion

The findings from animal experimentation and observations made upon a physical model are in accordance with the conditions was observed in the peripheral and insular capillaries of the pancreas in cases of chronic passive congestion. The results of the animal experiments, though they clearly show a lack of dye in the peripheral capillaries in partial venous obstruction, do not indicate that the flow under these conditions is nil in these channels. The aorta was ligated immediately when the injection was considered complete, thus stopping the circulation completely. The injection was considered complete when the dye began to appear in the efferent venules of the enlarged liver — indicating that it had passed through some of the capillaries and had entered the portal venous system. The portal vein in each case was blackened. If time had been allowed, in any degree of partial obstruction, for the blood to travel through the peripheral capillaries, some dye undoubtedly would have been seen in these vessels. The experiments indicate, however, that there is a lack of uniformity of flow in all parts of the pancreatic lobule, which is due to the peculiar anatomical structure of the circulatory pattern. The result is that there is ensured a maximum flow of blood through the islands in various degrees of obstruction, at the same time favoring conditions for stasis in the capillaries of the marginal zones of the primary lobules.

In simplifying the physical model, the flexibility of the blood vessels, their tapering structure, the number of capillaries and branches, and their conformity in size were sacrificed. A fluid of little viscosity was used as the circulating medium. As the results of the experiments with the physical model appear to parallel closely those of the animal experiments, they indicate that the pressure relationships are the major factors involved.

Summary

1. Animal experiments were done which indicate a lack of uniformity of the flow of blood in all parts of the primary lobules of the pancreas. This inequality of flow is based upon the anatomical

structure of the circulatory pattern, which favors a faster flow through the islands of Langerhans than through the peripheral capillaries.

2. The flow through the islands is not seriously embarrassed in considerable venous obstruction, whereas in the peripheral capillaries there is a tendency to congestion.

3. Experiments with a simple physical model, which appear to parallel closely the animal experiments, indicate that the pressure relationships are the major factors involved.

NOTE: We wish to thank Dr. C. Sidney Burwell of the Department of Medicine for making possible these experiments and for helpful suggestions, Dr. Ernest W. Goodpasture of the Department of Pathology for reviewing some of the microscopic slides, and Drs. T. D. Cope and H. C. Barker of the Department of Physics of the University of Pennsylvania for helpful criticisms.

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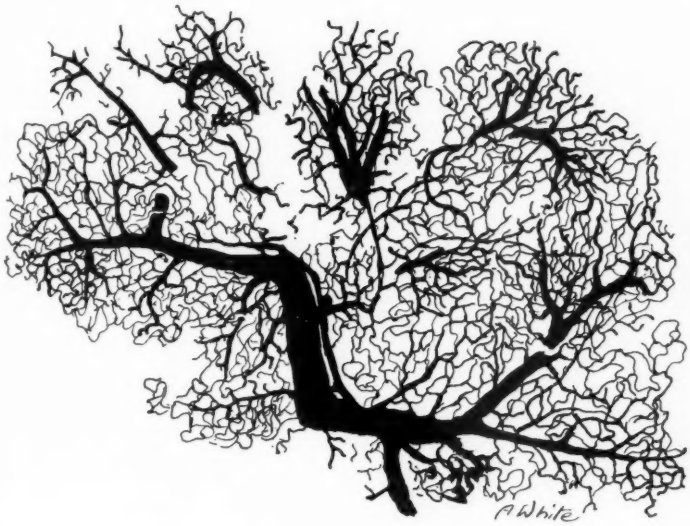
DESCRIPTION OF PLATES

PLATE 104

FIG. 1. Part of a pancreatic lobule. No venous obstruction. The arteries, veins and capillaries are uniformly filled with the black dye. Low power drawing.

FIG. 2. The appearance of the pancreatic lobule in partial venous obstruction showing arteries, veins and islands of Langerhans. Low power drawing.



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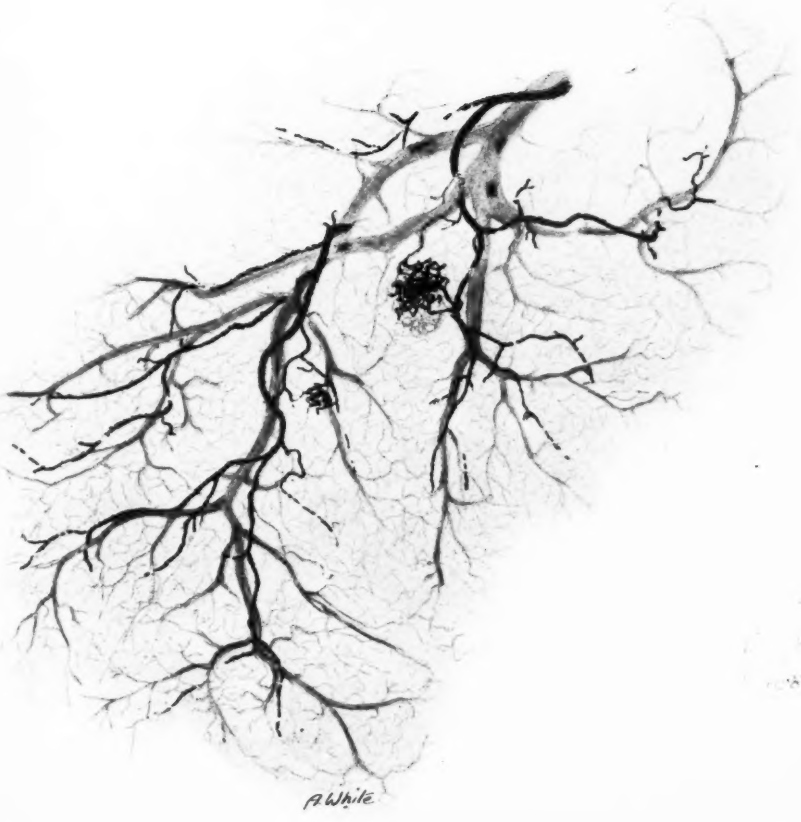

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Beck and Peterson Circulation in Pancreatic Lobule

PLATE 105

FIG. 3. The appearance of the lobule in complete venous obstruction showing arteries, veins and islands of Langerhans. Low power drawing.

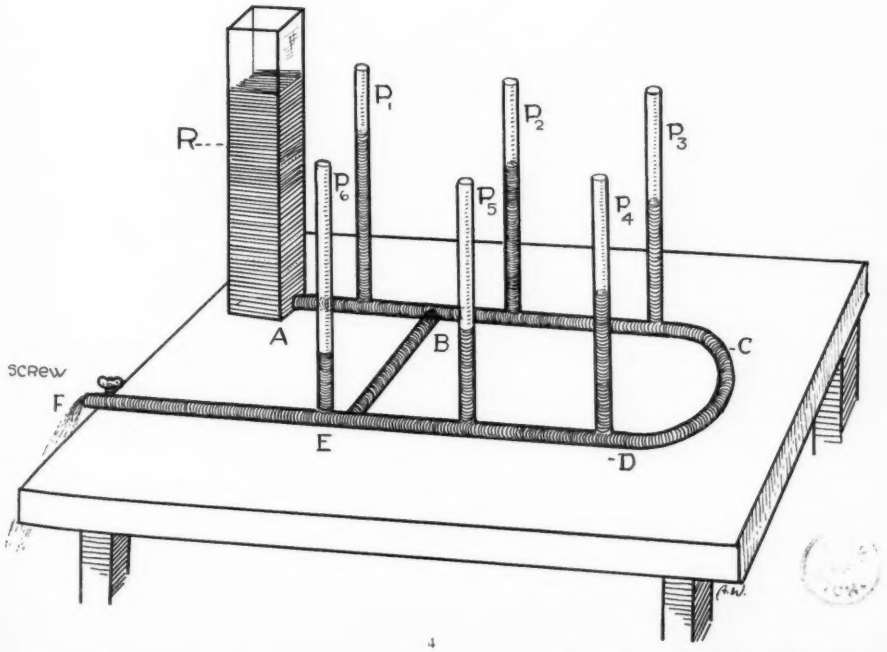
FIG. 4. A model constructed to conform closely to the circulatory pattern of the pancreatic lobules.

WORKING MODEL	ANATOMICAL REPRESENTATION
R = reservoir	Intralobular artery
AB = first part of proximal limb of U-tube	Afferent arteriole to island of Langerhans
AC = proximal part of U-tube	Small artery to acinar tissue
CD = bend of U-tube	Interacinar capillaries
DF = distal limb of U-tube	Small vein accompanying small artery to acinar tissue
BE = connecting tube	Capillaries in islands
EF = last part of distal limb of U-tube	Efferent venule of islands
F = outlet of distal limb (screw to vary obstruction)	Intralobular vein

P_1, P_2, P_3, P_4, P_5, P_6, are piezometer tubes

(Compare with Figs. 1, 2 and 3.)

3



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Beck and Peterson Circulation in Pancreatic Lobule

A SIMPLE METHOD FOR STUDYING THE CYTOLOGY OF THE INFECTIOUS MYXOMA OF THE RABBIT *

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It is well known that characteristic changes occur within host cells of animals and plants infected with certain filterable viruses. For this reason it seemed important to describe a simple and rapid method — in brief — a modified Wright's blood stain, that was found useful in studying the cells of the tumor produced by the virus of infectious myxomatosis of rabbits.

A small, covered Stender dish (about 1 inch high by $1\frac{1}{4}$ inches in diameter) was filled about half full with undiluted Wright's stain, and in another dish of the same size was placed about the same quantity of stain diluted one-half with distilled water. A tumor nodule of sufficient duration was selected and excised from the etherized animal. A thin slice of the tumor was laid flat on a thin 1 inch square coverglass and slowly dragged across its surface. This was quickly dropped, spread side down, into the dish of undiluted stain. It was important to avoid drying of the spread before it reached the stain, and yet to escape using tissue that was too moist. When the tumor was so moist that the cells did not stick to the coverglass, the piece of tumor was placed on filter paper until some of the serum drained away, or it was cut up with small curved scissors into a soft pulp and a little of this dragged across the coverglass. In order to obtain spreads of the ectoderm cells the outer layer of the epidermis was removed and cut up into a pulp with a fragment of moist, normal subcutaneous tissue taken from a nearby region.

A number of moist spreads were dropped into the same dish of stain where they were left from 1 to 10 minutes, or even longer, provided the dish of stain was covered to prevent evaporation. However, when it was not convenient to stain all of the spreads at once some of them were dropped into methyl alcohol where they were kept until needed. The coverglasses were removed one by one from

* Received for publication April 25, 1932.

the undiluted stain and placed in the dish containing the diluted blood stain. After 2 to 10 minutes they were removed one by one, blotted (not dried), passed rapidly through two dishes of absolute alcohol into xylol, and then into clean xylol, from which they were mounted in balsam on a slide.

In order to compare the preparations stained with Wright's with those stained by other methods, some moist spreads were fixed in methyl alcohol and stained with Giemsa's stain; others were fixed in one-third absolute alcohol plus two-thirds saturated aqueous corrosive sublimate and stained with methyl green and acid fuchsin or with methyl blue and erythrosin; and some were fixed with Zenker-formol and stained with hematoxylin and eosin. All of the methods furnished excellent preparations, but the simple Wright's blood stain was the most satisfactory and furnished the most differentiation.

By means of Wright's stain the greatly hypertrophied spindle and stellate cells showed with marked clearness. The numerous chromatin granules in the large nuclei of these cells, described by Aragao,[1] were sharply stained a deep purple and the hypertrophied true nucleolus a pinkish blue. The myxomatous material described by Hobbs[2] in these cells was stained slightly pink. The granular inclusion body in the ectoderm cells, described by Rivers,[3] was stained pink; the granules in the polymorphonuclear cells became a bright red, the basophilic granules in the mast cells a blackish purple; erythrocytes were orange; ingested red blood cells were a greenish blue and ingested polymorphonuclear cells showed as red granules and blue fragments of nuclei. Numerous rickettsia-like granules, probably the granules described by Lipschütz,[4] present in epithelioid cells and in hypertrophied monocytes of the tumor were stained a pinkish purple.

By the Wright's blood stain it was evident that the greatly enlarged spindle and stellate connective cells, as described by Hyde and Gardner,[5] with their hypertrophied hyperchromatic nuclei and myxomatous material were the cells that correspond to the malignant cells of mammalian tumors. It is probably from the presence of these cells that the lesion was designated as a myxoma, although the marked infiltration of polymorphonuclear leucocytes which takes place in the rabbit myxoma is not characteristic of tumors in general.

The ectoderm cells covering the lesion were not so clearly differentiated by the Wright's stain as by the method described by

Rivers; nevertheless, the somewhat granular pink material lying at one side of the nucleus was readily identified.

Aside from the stellate cells (myxomatous cells) the most striking characteristic of the tumor tissue, differentiated by the Wright's stain, was the numerous, large, flat, granular epithelioid cells scattered through the dermal and hypodermal tissue, especially in the region of blood vessels. These cells contained many small granules stained pinkish purple that were quite different from the red granules of the polymorphonuclear cells, the blue granules of the basophiles and mast cells, or the greenish blue ingested material. The granules in the various epithelioid cells were of different sizes and shapes, although those present in one cell were in general about the same size. In a single cell they varied from small round granules and short rods to more or less triangular-shaped granules. They were massed around the centrosome at one side of the nucleus, from which they scattered out to more or less fill the cytoplasm of the cell. They exhibited appearances strikingly similar to the rickettsia bodies, *Dermocentroxenus Rickettsia* or *Rickettsia Prowazeki*, but were more like the body Cowdry [6] described in heart water disease as *Rickettsia ruminantum*. These granules were present in cells of spreads prepared by the other methods described, but they were not so clearly differentiated by any of the other stains used as by the Wright's blood stain.

The cells which contained the granules belong to the group of hypertrophied and transformed mononuclear leucocyte (Lewis and Lewis[7]), and were usually of the epithelioid cell type, although the characteristically stained granules were also present in monocytes, hypertrophied monocytes, and macrophages. Other macrophages, containing ingested red blood cells and cellular débris, were often present in the same field. These cells contained some of the purple rickettsia-like granules, and the epithelioid cells occasionally had an ingested dead polymorphonuclear leucocyte, although as a rule the epithelioid cells did not contain ingested cellular débris.

While Wright's blood stain gave on the whole the most satisfactory results, some of the other methods differentiated some one structure even more clearly — for instance — with Wolbach's modification of Giemsa's stain the myxomatous material in the stellate cells appeared as a large pink body within the surrounding blue cytoplasm, and by Auerbach's method the chromatin granules in the hyper-

trophied nuclei of the malignant cells became deep blue and the nucleolus bright red. Giemsa's stain was an excellent one, except that the ingested material, the granules of the polymorphonuclear leucocytes and the granules of the epithelioid cells, were all stained red and so not easily differentiated from one another.

In the earliest tumors studied (48 hours) neither the spindle cell nor the macrophage exhibited marked hypertrophy, although the nuclei of many of the spindle cells were already somewhat granular. Both types of cells frequently contained a number of purple granules so that they were not so strikingly different as in the larger tumors.

As the tumor increased in size the number of stellate cells (malignant cells) increased. These cells became much larger than the normal connective tissue cells, their nuclei hypertrophied and became granular and a diffuse staining material called myxomatous material became evident in the cytoplasm of a number of them. Most of these cells were free from the purple cytoplasmic granules, although even in the most advanced tumor examined occasionally a malignant cell was found with purple granules in the cytoplasm.

Coincident with the increase in size of the tumor there occurred an increase in number of monocytes, hypertrophied monocytes, macrophages and epithelioid cells. The epithelioid cells became larger and the number of granules became greatly increased, so that in the large tumors hypertrophied epithelioid cells with their cytoplasm filled with the small purple granules were frequent.

In an effort to gain an understanding of the nature of the small cytoplasmic granules many other types of tissue, including granulation tissue, rat and human tumors, and normal rat, rabbit and chicken skin, subcutaneous tissue, spleen, liver and lymph node, were studied by means of spreads fixed and stained with Wright's blood stain. While many monocytes, clasmatocytes and epithelioid cells were found in these tissues they did not exhibit the small purple granules characteristic of the infectious myxoma of the rabbit.

Just what the nature of the granules is has not been determined. They resemble bodies described as rickettsia in size, location in cells and in certain but not all staining reactions. Lipschütz claims to have determined that they are not rickettsia bodies. It is possible that they are accumulated protein material, arising from the infiltration of the tumor with myxomatous colloid, for Lewis [8] found that chick embryo cells grown in a medium containing white of egg or

plasma became full of small, more or less even-sized granules called albumin granules. Hobbs suggested that the granular cells might be hypertrophied mast cells, but their differential staining with Wright's blood stain shows that the granules are not similar to those of the polymorphonuclear or of the basophilic leucocyte.

Whatever the nature of these bodies may be they were found to be characteristic of the many tumors of the infectious rabbit myxoma studied for a period of several years, and to be clearly demonstrated by means of moist spreads of the lesion fixed and stained by means of Wright's blood stain.

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SCIENTIFIC PROCEEDINGS OF THE THIRTY-SECOND ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

PHILADELPHIA, PENNSYLVANIA

April 28 and 29, 1932

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

Voted to elect the following officers for 1932–1933:

President	E. T. Bell
Vice-President	O. T. Avery
Treasurer	F. B. Mallory
Secretary	Howard T. Karsner
Incoming Member of Council	N. C. Foot
Assistant Secretary	Robert A. Moore

ABSTRACT OF MEETING OF THE COUNCIL

Voted to elect the following new members:

Donald C. Beaver	James Watson Kernohan
Virgil H. Cornell	Frank W. Konzelmann
Theodore J. Curphey	George F. Laidlaw
R. Philip Custer	Samuel A. Levinson
Vincent J. Dardinski	John Loesch
A. Hobson Davis	John Franklin Noble
Cornelia M. Downs	Gorton Ritchie
Marcos Fernan-Nunez	Andrea Saccone
Irving Graef	Tom Douglas Spies
Paul Henry Guttman	Evan Lee Stubbs
Charles H. Hitchcock	M. Juanita Thompson
Lloyd R. Jones	Leslie T. Webster

John W. Williams

Voted to accept with regret the resignations of Dr. M. A. Barber and Dr. Channing Frothingham.

Voted to record with deep regret the deaths of Dr. C. G. Bull, Dr. V. A. Moore and Dr. A. S. Warthin.

Voted to adopt as the topic for the symposium for 1933 the subject of Pneumonia.

Voted that no papers on lymphatic tumors be accepted for publication in the American Journal of Pathology unless the tumors have been submitted to the Registry.

Voted to meet in Washington, D. C., on May 9 and 10, 1933, in conjunction with the meetings of the Congress of American Physicians and Surgeons.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A Preliminary Report on the Effect of Shaking as Applied to the Vernes Test for Syphilis.

Adelaide B. Baylis (by invitation), New York City.

Abstract. In a series of 100 selected specimens of blood serum the classical flocculation test of Vernes was carried out and, at the same time, a duplicate test on the identical specimens by a modified technique, in which the tubes were mechanically shaken for 15 minutes immediately after the addition of the perethynol to the serum. The shaking was found to increase the sensitivity without evident danger of false positive reactions. Specimens giving a slight degree of flocculation in the classical test were found to give a more decisive result when shaken, some of them now giving no flocculation at all, and others a higher reading which could be regarded as more definitely positive in character. Shaking did not alter the readings on the control tubes in any instance.

The results indicate that physical agitation increases the opportunity for surface contact and reaction between the colloidal particles of the antigenic substance and the reagin of the serum, and suggest that analogous shaking may be of similar value in other serological reactions.

Discussion

(Dr. Reuben L. Kahn, Ann Arbor.) I was glad to hear the presentation by Miss Baylis on the problem of shaking in precipitation. When we first observed that shaking hastens the precipitation reaction in syphilis, we made quantitative studies of the effect of the speed and the duration of shaking on the reaction. We observed that when the shaking speed was excessively rapid or the duration too prolonged, there was a tendency for the precipitate to become emulsified, so that clear-cut particles would become indefinite. We observed in connection with our precipitation reaction that a 10 minute shaking period, for example, would often change a strongly positive reaction to a weak reaction. Since Miss Baylis speaks of shaking 15 minutes, it seems to me that perhaps a shorter period of time might give better results.

(Miss Baylis.) In these experiments I started shaking at a period of from 1 minute up to the entire period of 4 hours. From these preliminary studies, which are not reported, I was able to decide that shaking for 15 minutes gave a suitable reaction. After 15 minutes the reaction was rarely changed, and I also found in these tests that the shaking for 15 minutes sometimes served to change an otherwise indefinite result to a definite answer, either on the positive or the negative side.

(Dr. Kahn.) I may say that 15 minutes shaking from a practical point of view is a difficult problem. Some workers consider the 3 minute shaking period in our test as too lengthy.

(Miss Baylis, closing.) I may also add that I tried different speeds.

A Study of Pathogen-selective Cultures in Relation to Vaccine Therapy. Fred Boerner and Myer Solis-Cohen (by invitation), Philadelphia, Pa.

Abstract. The Solis-Cohen pathogen-selective method for preparing autogenous vaccines from mixed cultures is based upon the assumption that organisms capable of growing in the fresh, whole, coagulable blood of the patient are those which are most pathogenic for that individual. It consists of two simultaneous inoculations of the material to be cultured, one in a rich medium, such as Rosenow's brain broth, and the other in the patient's fresh, whole, coagulable blood *in vitro*. After a preliminary incubation of 24 hours, both cultures are examined and the organisms present in each are studied for identification. The organisms which appear in the blood are those selected to predominate in the vaccine. Four hundred pathogen-selective cultures from 150 patients were studied. In approximately one-third the results were identical in both the broth and the blood. In one-fourth only certain of the organisms present in the broth grew in the blood. In one-fourth none of the organisms present in the broth grew in the blood. In 11.5 per cent the organisms that grew in the blood failed to grow in the broth, and would therefore have been missed by the ordinary methods of culturing. Streptococci and staphylococci grew most frequently in the patient's blood, the hemolytic strains showing the highest percentage of the former and the aureus of the latter. Few of the Gram-negative cocci or of the non-pathogenic Gram-negative bacilli grew in the blood.

The pathogen-selective method of culturing has also been found useful as an aid in isolating such organisms as the streptococcus from mixed infections and contaminated material.

A Study of Bacterial Hypersensitiveness, with Special Regard to Its Value as Indicating Pathogenicity, and with a Comparison of Cutaneous, Intracutaneous and Subcutaneous Tests and of Their Relative Values for Suggesting Appropriate Vaccine Dosage. Myer Solis-Cohen (by invitation), Philadelphia, Pa.

Abstract. In a study of the reactions produced by intracutaneous injections of dead bacteria (with their soluble toxins) obtained from patients, those produced by organisms that grew in the patient's fresh, whole, coagulable blood *in vitro* were compared with those produced by organisms that were killed by the patient's blood. No correspondence was observed between ability to grow in the patient's blood and ability to produce a reaction when injected intracutaneously. It may be inferred therefore that the intracutaneous skin test does not differentiate organisms that are capable of infecting the patient from those against which he possesses good resistance, and consequently is not a reliable means of determining which organisms in a mixed culture should be included in a vaccine.

A comparison of simultaneous intracutaneous and cutaneous tests with the same vaccine showed both to be positive in 26 per cent of the cases, both to be negative in 10 per cent, and the intracutaneous to be positive and the cutaneous negative in 64 per cent, the former being 3 plus in 26 cases, 4 plus in 10, and 5 plus in 4. In no case was the cutaneous test positive and the corresponding intracutaneous test negative. The intracutaneous injection therefore furnishes a much more accurate test for hypersensitiveness than does the cutaneous inoculation, which moreover cannot be regarded as reliable.

Reactions from intracutaneous and subcutaneous injections of the same vaccine were compared, those from the latter being divided into general, focal and local. The absence of general and focal reactions after intracutaneous injection makes one question the efficacy of this method for therapeutic administration. The intracutaneous injection was given as a test, prior to the therapeutic subcutaneous injection, the degree of reaction affording a guide in determining the appropriate initial therapeutic dose. In most cases the same dose was given intracutaneously and subcutaneously, but when the reaction from the former was very marked, a smaller amount was injected subcutaneously. In 61 per cent of the cases the reactions from the corresponding intracutaneous and subcutaneous injections were of equal intensity — in 13 per cent the intracutaneous was the more severe, and in 26 per cent the subcutaneous was the more severe. An initial therapeutic injection with dose based upon the reaction to a preceding intracutaneous test produced a reaction that was not severe in 87 per cent of the cases. The intracutaneous test may therefore be regarded as a fairly safe and accurate aid in determining the initial therapeutic dose, subsequent doses being satisfactorily determined according to the reaction produced by the immediately preceding subcutaneous injection.

Discussion

(Dr. Marcus W. Lyon, South Bend.) What are the therapeutic results obtained with these selective cultures?

(Dr. Solis-Cohen.) They are quite good; they are much better in cases that have the vaccine over a fairly long period of time — more than four or five doses. We have obtained good results in asthma, in rheumatism, and in sinus infections. The vaccine was often given before an operation on the sinus or tonsils, half the course before the operation and half afterwards, and the patients did much better than those who did not receive the vaccine. In pyelitis and in many other conditions the results were quite favorable.

(Dr. Max M. Strumia, Philadelphia.) Were any controls run in connection with the therapeutic applications, as well as the skin tests?

(Dr. E. T. Bell, Minneapolis.) I should like to have Dr. Solis-Cohen explain a little more fully the basis of the assumption that the organisms that grow in the blood are the ones responsible for infection. One would expect to find immune bodies in the blood that would inhibit the growth of the organism responsible for a chronic infection.

(Dr. E. C. Rosenow, Rochester, Minn.) I am rather puzzled over the large number of different varieties of organisms that are regarded as pathogenic and am wondering whether the method is considered sufficiently reliable to attach etiological significance to all of the various organisms that grow in the blood of any particular case, or only to some. The cause of different diseases is usually assumed to be one organism, and not a mixture. In our own work this assumption is borne out by the fact that with few exceptions only one organism, usually a streptococcus, localizes in animal tissues corresponding to those affected in the patient from whom isolated, following intravenous injection of primary cultures from various atria of infection. Specific vaccines prepared from the strains thus "proved guilty," when given in appropriate dosage, have been helpful in many cases.

(Dr. Frank B. Lynch, Philadelphia.) I should like to ask if any comparative tests were run on the cultures, using normal blood instead of the patient's blood.

(Dr. Ward J. MacNeal, New York City.) I should like to remark that the suggestion of the use of a patient's blood as a culture medium to select from mixed material the organism pathogenic for that individual patient requires further evidence in order to support such a conclusive identification. I should like also to call attention to the fact that such a method is extremely helpful in attempting to obtain in cultures an organism which would otherwise be missed, and I think an outstanding example is Ducrey's bacillus of chancroid, of which it is extremely easy to obtain a culture by using the patient's blood or the bacteriologist's blood or sheep's blood for the primary culture, but it is extremely difficult to obtain a culture without blood.

(Dr. Solis-Cohen, closing.) First, in regard to the theory: about fifteen years ago in an endeavor to find out why the chicken and pigeon are immune to pneumococcic infection, while the mouse and rabbit are susceptible, the various known antibodies were studied by Heist, Solomon Solis-Cohen and Myer Solis-Cohen, to see whether they were present in the chicken and pigeon and not in the mouse and rabbit. It was found that the serum of a pigeon does not differ in its action upon pneumococci from the serum of a mouse or rabbit. In the whole, fresh, uncoagulable blood of the pigeon, however, a bactericidal factor was found present which is absent from the whole, coagulable blood of the mouse or rabbit. Later Matsunami and Kolmer, employing our method, found a similar bactericidal action against meningococci in the whole, coagulable blood of the resistant rabbit that was absent from the whole blood of the susceptible mouse. Similar parallels were observed between the varying susceptibility of laboratory animals to other organisms, with the formation of the hypothesis that when small numbers of bacteria are planted in fresh, uncoagulated blood, only those bacteria grow and multiply which are pathogenic for the species from which the blood is drawn. Upon this was based the assumption that organisms capable of growing in the fresh, whole, coagulable blood of an individual are those which are most pathogenic for him.

In regard to the therapeutic results, Lowe of Liverpool published 100 cases of rheumatism treated by this method, and others have reported excellent therapeutic results.

As regards therapeutic controls, the hospitals are full of cases treated with vaccine prepared in the ordinary manner.

As regards skin controls, the organisms were not tested on normal people, but on their own hosts.

Speaking about the pathogenic significance outside of the streptococci, I think this is illustrated very well in pyelitis and cystitis, conditions in which vaccine treatment is commonly of little value. Ordinarily the colon bacillus alone will grow in cultures from the urine, but when the urine is inoculated in the patient's whole, coagulable blood, in some cases the colon bacillus persists, while in other cases it disappears, and is replaced by streptococci or staphylococci, which also are present in cultures from the nose and throat of the same patient. In two of the cases charted the colon bacillus from the feces which grew in Rosenow's medium was killed off by the blood. This does not prove that the organisms growing in the blood are pathogenic, and the others are not, but it is very suggestive.

Dr. Lynch asked if the work was done with normal persons as well as with these patients. I reported before this Association eleven years ago concerning large groups of normal persons who were tested with colon bacilli, streptococci and meningococci. The meningococcus from the spinal fluid of patients with cerebrospinal meningitis grew in most of the bloods of the people tested, but the meningococcus from the throats of carriers grew in only a few individuals, the conclusion being drawn that the spinal fluid strains of meningococci are much more virulent for man than are the carrier strains, and that the minority of men, whose blood permits the rapid growth of carrier strains, are more likely to develop meningitis after exposure to a carrier. Dr. Heist, who did the work, found that the carrier strains always grew in his own blood, which he used as a control. Before the paper was read, Dr. Heist died of meningococcic meningitis, despite all efforts to save him.

THE RELATION OF SENSITIZATION OF THE FLAGELLA AND SOMATA OF THE TYPHOID BACILLUS TO PHAGOCYTOSIS. Stuart Mudd, Balduin Lucké, and Max Strumia, Philadelphia, Pa.

Abstract. Antityphoid sera containing both somatic and flagellar agglutinins, and sera containing either only somatic, or only flagellar, agglutinins have been tested for their power to promote, *in vitro*, phagocytosis by macrophages and polymorphonuclear leucocytes. The somatic antisera were prepared by injection into rabbits of an aflagellate variant of the typhoid bacillus (strain o 901); the flagellar antisera were prepared by adsorption of the complete antisera with the aflagellate variant. All three types of sera were found capable of promoting phagocytosis of typhoid bacilli both by macrophages and by polymorphonuclear leucocytes. Digestion of the typhoid bacilli occurred rapidly within both types of phagocytes. Such digestion unless adequately taken into account may lead to fallacious results in phagocytosis studies. From this work it appears that the tendency in the literature to regard flagellar antibodies as of no value as defensive factors is unwarranted.

LOCAL IMMUNITY AND THE LOCAL FORMATION OF ANTIBODIES. Paul R. Cannon and (by invitation) F. L. Sullivan, Chicago, Ill.

Abstract. Evidence is submitted suggesting a correlation between locally increased resistance, local mobilization of cells of inflammation, particularly macrophages, and local formation of specific antibodies (agglutinins).

Rabbits were injected repeatedly intracutaneously in the same area with a formolized vaccine of *B. paratyphosus B.* At various intervals this area, a corresponding area on the opposite side, blood serum, liver, spleen, and other organs were extracted under comparable conditions with glycerol-saline solutions in order to determine the relative concentrations of agglutinin.

Specific agglutinins were found in relatively high concentration in the locally treated area before they could be detected in significant amounts in the blood serum or in other organs. Non-specific inflammation in the skin of the opposite side of an animal locally vaccinated did not lead to a concentration of antibodies in the inflamed area.

These findings tend to substantiate the view that a locally increased resistance may be obtained by measures which secure a local concentration of phagocytes and specific antibodies.

Discussion

(Dr. B. J. Clawson, Minneapolis.) This work of Dr. Cannon's brought out two very fundamental considerations: the first, that the mechanism of holding organisms at the point of the allergic area is probably due to antibody rather than to a mechanical mechanism, and the second, that if vaccine is applied to diseases where we want a general immunity, the intravenous method should be used, rather than the intracutaneous or subcutaneous methods.

PHENOMENON OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES IN THE TREATMENT OF MOUSE SARCOMA 180. Gregory Shwartzman and (by invitation) Nicholas Michailovsky, New York City.

Abstract. The phenomenon of local skin reactivity to bacterial filtrates described by one of us (Shwartzman) in 1928 was later also reproduced in the liver, kidney (Shwartzman); testis, intestines, lymphatic glands, lungs, thymus, guinea pig liposarcoma (Gratia and Linz); stomach (Karsner, Ecker and Jackson); and knee joints (Moritz and Morley). It was elicited with a great variety of microörganisms (Shwartzman), and also with vaccine virus as the preparatory factor (Gratia and Linz). The animals in which the phenomenon was observed were rabbits, horses, goats (Shwartzman); and guinea pigs (Gratia and Linz). It could not be reproduced in mice and rats (Shwartzman). Assuming that malignant tumors may be of parasitic etiology (Gratia and Linz), it was thought that the hypothetical virus should then be capable of inducing a state of reactivity in the tumor tissue and thus render it susceptible to reacting factors in the blood stream. Five guinea pigs bearing liposarcoma were injected intravenously with *B. coli* culture filtrate. Two guinea pigs which died 24 hours later and two killed 48 hours later showed at autopsy hemorrhagic lesions in the tumor tissue and no lesions in other organs. The fifth guinea pig was left alive for further observations. Guinea pigs were selected because of their susceptibility to the phenomenon of local skin reactivity to bacterial filtrates.

Since it was deemed important to determine whether or not this phenomenon could be reproduced in transplantable tumors in mice the effect of bacterial filtrates upon Mouse Sarcoma 180 (Crocker Institute) was studied by the authors. This strain of sarcoma was selected on account of its high growth energy and malignancy. The bacterial filtrate employed was of high phenomenon-producing potency, as previously determined in rabbits (Shwartzman), namely "agar washings" filtrate of Meningococcus 44 D group I (*i.e.*, filtrate #1700 containing 1350 reacting units per cc.).

By means of single or repeated intravenous or intraperitoneal injections of this filtrate, it was possible to elicit prompt severe hemorrhage in Mouse Sarcoma 180. The first appearance of the effect resembled very closely the phenomenon of local skin reactivity to bacterial filtrates. The hemorrhage brought about progressive damage of the tumor, which was followed either by complete elimination of the tumor and healing in a high percentage of mice, or by a striking regression with further slow reappearance of tumor growth. The effect upon the tumor appeared to be selective, inasmuch as the intravenous and intraperitoneal injections of the filtrate produced no hemorrhagic lesions in other organs of the mouse.

Although there appeared to be a close resemblance between this reaction and the phenomenon of local skin reactivity to bacterial filtrates, it does not necessarily mean that a virus must be responsible for the state of reactivity of the tumor cells to reacting factors introduced via the blood stream. Studies on other possible explanations are on the way. Observations reported are considered of interest because there appears to be a remarkable selective destruction of a tumor which is of a high malignancy and of rapid growth, and which shows spontaneous regression only very rarely, and also because being obtained in mice, these observations offer an opportunity for further studies on the relation of the "phenomenon of local skin reactivity to bacterial filtrates" to problems of tumor growth.

Discussion

(Dr. Howard T. Karsner, Cleveland.) There is in this reaction a series of interesting changes demonstrated by the microscopic examination of tissues, which may perhaps throw some light upon the nature of the process. In our work on the rabbit stomach, and that of Dr. Moritz and his associates on the rabbit knee joints, we were impressed by the fact that the inflammation produced does not differ from the ordinary exudative variety of inflammation except that the amount of hemorrhage is striking in all situations. This leads one to suppose that damage to the blood vessels is severe. The anatomical demonstration of that damage to the blood vessels is difficult, but in the rabbit knee joint it is possible to show actual necrosis of the walls of the vessels. These examinations were made on animals treated in the regular way by injection under the skin, and then subsequently by intravenous injection. The first injection in the skin or other tissues produces of itself, apparently as a result of the irritative nature of the filtrate, a slight degree of inflammation, and this is markedly augmented when the intravenous injection is given. If the key part of the picture is damage to the blood vessels, — and whether that can be demonstrated morphologically after the first injection or not seems to be of little significance, — the fact that in some situations we can observe after the second injection actual damage to the blood vessels, and the further fact that hemorrhage is a prominent part of the reaction, leave no ground for doubt that vascular damage is severe, whether microscopically demonstrable or not. A sarcoma is a richly vascularized tumor, and it would seem, in these examples which Dr. Shwartzman has shown to us, that the damaging effect of the filtrate on the vessels has led to a profound hemorrhage, interference with the nutrition, and a consequent regression of the tumor. Since there is some interrelation between this reaction and the action of mocassin venom, the question naturally arises as to whether or not snake venom would have an effect upon the mouse sarcoma similar to that of the Shwartzman filtrates.

(Dr. Shwartzman, closing.) I only want to say in reply to Dr. Karsner that his suggestion to study the effect of snake venom is very interesting. However, the striking feature of the results reported is the selective effect of the meningococcus filtrate upon sarcoma 180, while the snake venom would presumably act as a general vascular poison. Of course, on the other hand, it is possible that the tumor blood vessels may prove to be more susceptible than the normal blood vessels to any vascular poison.

THERAPEUTIC APPLICATION OF BACTERIOPHAGE IN STAPHYLOCOCCUS BACTEREMIA. W. J. MacNeal and (by invitation) Frances C. Frisbee, New York City.

Abstract. A bacteriophage highly potent against staphylococci found in infections of the blood stream has been used for treatment of staphylococcus bacteremia, chiefly by intravenous injection, but also by local application to wounds and by subcutaneous injection. In a series of fifteen patients there have been eight deaths and seven recoveries. The treatment is not a simple matter, and the course of the disease leading to recovery is prolonged and beset with many dangers which may be fatal. The careful and intelligent use of bacteriophage may be expected to assist somewhat in the treatment of this very grave condition.

Discussion

(Dr. Max B. Lurie, Philadelphia.) Was the effect of the bacteriophage used in these cases tested on the staphylococci isolated from the blood of the patients?

(Dr. Reuben L. Kahn, Ann Arbor.) I might mention in this connection that we have had at the University of Michigan Hospital at Ann Arbor six cases of staphylococcus septicemia with multiple abscesses, three of which recovered following bacteriophage therapy. One did not completely recover; this was a girl 16 years old who developed osteomyelitis. We have also tried the use of bacteriophage in the treatment of osteomyelitis. A report based on ten cases is to appear soon. The conclusions of the surgeons seem to be that when the osteomyelitis is due to a pure culture of staphylococcus with freedom from other organisms, such as *B. pyocyaneus* and streptococci, the effect of bacteriophage is favorable.

Another point of interest is that in chronic cases of osteomyelitis (as shown by Albee) we have found bacteriophage in the wounds, especially with the Orr closed wound method of treatment. Some have expressed the likelihood that the therapeutic effect that Orr believes takes place as a result of his method of treatment might be due to the presence of bacteriophage. I think, as Dr. MacNeal pointed out, that while one cannot be over-enthusiastic about this method of treatment, yet, in a condition as severe as staphylococcus septicemia, especially with multiple abscesses, the use of bacteriophage would seem justifiable.

(Dr. Gregory Shwartzman, New York City.) Between 1923 and 1927, I was very much interested in the therapeutic application of bacteriophage. We divided our cases into two groups, those receiving the bacteriophage intravenously, and those receiving it locally. As Dr. MacNeal pointed out in his charts, the intravenous injections have very unpleasant effects. They invariably produce chills and rather severe shock. These effects may be attributed to the presence of a great deal of toxic substances in the bacteriophage culture.

I think that the outcome of the staphylococcus septicemia depends very much on the focus from which it originates. I agree with Dr. MacNeal that staphylococcus septicemia, following osteomyelitis, is usually fatal, but some staphylococcus septicemias originating from phlebitis may improve spontaneously. Some time ago, we had a case of extremely severe staphylococcus septicemia following a phlebitis of the uterine veins. The patient recovered, however, without any treatment.

In view of the unpleasant consequences of intravenous bacteriophage therapy, we gave it up entirely and studied only the local effect of bacteriophage. Inasmuch as there is no bacteriophage for pyogenic streptococci, its application is limited only to staphylococcus and *B. coli* infections. The staphylococcus infections seem to yield quite well to the local application of bacteriophage. Pyelitis is another lesion which also seems to be influenced favorably by the bacteriophage if the treatment is kept up consistently for a certain length of time. There remains the question whether or not the effects obtained are due to the bacteriophage as such, or to the bacterial toxic substances included in the filtrates. I think that the local and general vaccination induced by the use of such filtrates plays a more important rôle than the lytic effect. In support of this point of view is the fact that in most instances cultures obtained from the bacteriophage treated wounds may grow quite profusely and prove to be resistant to the bacteriophage, in spite of the fact that the patients are doing better. One is more inclined, therefore, to the view that the effects are due either to the stimulation of the natural defense mechanism, or, as Dr. MacNeal thinks, to a change in the bacteria themselves, possibly in their antigenicity and pathogenicity.

(Dr. Preston Kyes, Chicago.) It seems to me that consideration of these clinical results following the intravenous injection of bacteriophage raises, at once, the question of the specificity of the reactions induced.

It is well established that the intravenous injection into man of particulate matter, or of reagents which induce the intravascular formation of particles, provokes the type of chill reaction seen in the cases reported. It is also recognized that when such non-specific reactions are provoked, they may be attended by a profound modification of an existing infection. I see nothing in the results reported in this particular paper which has not been claimed by those who have used intravenous injections of milk, of raw serum, of peptone, of suspensions of killed heterologous bacteria, and so on in the non-specific treatment of generalized infections.

Without questioning the favorable clinical results in the group of cases presented, I would question the deduction that the action of the bacteriophage in these cases is shown to be that of a specific reagent.

(Dr. MacNeal, closing.) Obviously the paper was considerably abbreviated in presentation, and I hope you will take that into consideration.

In regard to the assumption of specificity of the bacteriolytic agent for the bacteria, that was not based on the behavior of the patient, but on test tube experiments. In each instance a culture of the staphylococcus was treated by the bacteriophage, and the culture was completely sterilized by it in the test tube, so we have evidence that there is some reaction between the bacteriophage and the microbe. It seems to me that nobody who is familiar with the work of Gratia can doubt that there is an agent effective against staphylococci. I had assumed I could pass over that. We have insisted in each instance that a culture should be tested out against bacteriophage in the laboratory, and so far, in staphylococcus bacteremias, we have not come across one which is not susceptible to that agent. The thing I wish to discount is the assumption that such a phenomenon necessarily occurs also in the human body. I do believe, however, that some part of this influence may remain active in the body of the patient.

In regard to the osteomyelitis cases, I may say I purposely omitted any discussion of chronic osteomyelitis. I think Miss Patterson and Dr. Albee, some

years ago, called attention to the influence of bacteriophage in the Orr treatment of osteomyelitis, and I think the bacteriophage has been used as a routine by Dr. Albee in the past three years; I thought it was unnecessary to mention that.

About the question of the shock, I think part of the shock may be due to foreign protein and on account of that we have eliminated the use of the broth preparation in intravenous work. We are now using a preparation which is almost completely protein-free. This does not give any shock in animals not infected with staphylococcus, and it does produce an effect in animals which are infected with staphylococcus. We are of the opinion that this agent is without a shocking effect on persons without a staphylococcus infection. A person who has a staphylococcus blood stream infection gets a shock. In our experience, one has to push the dosage of the phage to the point of shocking the patient to get favorable results. I do not think it is necessary to cause a chill for forty minutes; that is too long, but we feel our way carefully until we get a rise in temperature or a chill, or both, and then we pause to let the patient rest, but not too long, because the bacteria have usually not been eradicated, and we must begin with the phage again after a brief rest.

The question of the use of the other agents which were mentioned, foreign proteins: I must confess I would welcome any particulate agent that could be used in the treatment of bacteremias with such microörganisms as staphylococci and streptococci, and that would bring about a reaction from which recovery might result. Anyone who utilizes fatally stricken human beings as experimental animals (and that is what we are doing) gets a sort of clinical view in which he desires to get the patient well. I suppose that is unscientific, but if any one would show me that the injection of milk would get a person with bacteremia well, I would be glad to use it. In our experience four years ago we hoped that a coccemia would turn out to be a streptococcus bacteremia, because we thought there was some hope of the patient's recovering spontaneously, whereas with the staphylococcus we had very little hope. Now we hope it will be just the reverse. We think we have changed the prognosis a little bit. One of the prominent surgeons in New York, Charles Gordon Heyd, President of the State Medical Society, told me that the use of staphylococcus bacteriophage has altered the prognosis of postoperative infections.

CORNEAL REACTIONS TO BACTERIUM GRANULOSIS AND OTHER MICROÖRGANISMS. P. K. Olitsky, R. E. Knutti and (by invitation) J. R. Tyler, New York City.

Abstract. In an attempt to simulate in animals human trachomatous pannus, intracorneal inoculations of 14 varieties of bacteria and various other materials were made. The cornea of the rabbit was found to be highly sensitive to the action of injected bacteria. The lesions varied from insignificant transient changes to severe, destructive panophthalmitis. Animals that received the same organism showed like changes. Only *Bacterium granulosis* induced early, uncompleted and enduring lesions which resembled human trachomatous pannus. This effect was similar in rabbits and in monkeys.

Discussion

(Dr. E. C. Rosenow, Rochester, Minn.) I should be interested to know whether the culture of *B. granulosis* which had specific effects had been under

cultivation on artificial media for a long time or whether it had been isolated shortly before from experimental animals.

(Dr. N. W. Popoff, Rochester, N. Y.) In connection with the work of Watanabe on non-specific inclusions produced by various substances I should like to ask whether controls with heat-killed bacteria, bacterial filtrates, other bacterial toxins and human serum were used in his experiments.

(Dr. Knutti, closing.) In answer to the question about toxins, no toxin has been demonstrated for *B. granulosis* itself. There were no filtrates of *B. granulosis* used; only the heat-killed organisms were injected intracorneally.

In regard to Dr. Rosenow's question, as to the source and age of these cultures: some of the cultures had been isolated from cases of trachoma up to two years before inoculation; others were fresh cultures. No difference in effect was discernible when old or young strains were injected.

REPORT OF THE LYMPHATIC TUMOR REGISTRY FOR THE YEAR 1931. George R. Callender, Washington, D. C.

Abstract. During the first four years, 1925–1928, inclusive, 100 cases were received; during the fifth year, 50 cases; during the sixth year, 85 cases, and during the last, or seventh year (1931), 60, making the present total 295 cases.

Of this group of 295, 50 are not lymphatic tumors. A considerable number of the cases are not entirely suitable for study because either inadequate material, tissues and blood smears, or inadequate data, have been furnished. Approximately 65 per cent are of some value for study, but only about 50 per cent of the total number have sufficiently complete records and adequate material. Because of poor follow-up, the most valuable cases from the standpoint of the Registry have been completed cases in which history is adequate, autopsy has been done and material furnished from the biopsy, autopsy, or both. Information with reference to radiation treatment, its kind and effect, is very meager. What few data have been accumulated indicate that the reaction of the tumor to radiation is of considerable importance.

It is believed that the members of this Association can find many cases in their files which are complete and the Committee urges that as many of such completed cases be sent to the Registry as possible, irrespective of whether they offer difficulties in diagnosis or not. The Committee further recommends that no case or cases of lymphatic tumors be accepted for publication in the American Journal of Pathology unless these cases have been placed in the Lymphatic Tumor Registry. A study of certain groups of the tumors is presented as a part of this report. It will be published in a later number of the Journal.

ON THE NATURE OF THE "HYALINE" MEMBRANE IN THE LUNGS. Sidney Farber, Boston, Mass.

Abstract. A study was made of the occurrence, composition, and mechanism of formation of the hyaline membrane, which has been generally considered pathognomonic of influenzal pneumonia. Membranes, indistinguishable in appearance and staining reactions from those seen in influenza, were repeatedly found in the lungs of young children, usually in association with streptococcus bronchopneumonia. No evidence of influenza could be found in these cases. Identical membranes were seen in the mediastinum in the presence of mediastinal emphysema and mediastinitis, and in the lungs of newborn infants who had aspirated amniotic sac contents after intrauterine asphyxia. Staining reactions

show that the membrane contains no fibrin. A high percentage of the membranes studied contain fat in large amounts. It was possible to produce characteristic "hyaline" membranes in the lungs of dead animals subjected to forceful artificial respiration while autolyzed purulent material was being injected into the trachea. From histological and experimental studies, it is concluded that the membrane is formed by the mechanical action of forcefully inspired air upon a viscous material in the air spaces. The viscous material is pressed against the alveolar walls in membrane formation and surrounds bubbles of entrapped air. Under similar mechanical conditions of dyspnea in the presence of semifluid material in the air passages, membranes identical in form and position, but not in staining reactions, may be found in a variety of circumstances. The composition of such membranes depends upon the material which happens to be in the air spaces at the time. The term *dyspneal* membrane is suggested as the most descriptive and appropriate designation for this histological picture.

Discussion

(Dr. George R. Callender, Washington.) I should like to ask Dr. Farber whether he found that the epithelial lining of the alveoli was or was not desquamated beneath this membrane.

(Dr. Farber.) We have found necrotic alveolar walls with desquamated epithelium in some cases, but more often the walls were intact.

(Dr. Callender.) The reason I asked was that I felt in going over the influenza lungs and also the lungs of ordinary pneumonia cases from the war period that early there was a rather thin exudate, and then following the desquamation of epithelium, a denser exudate was thrown out. I have also had some of the inspiration cases in babies, and in those occasionally have found small strands of fibrin, but as a whole, they were like those you have shown.

(Dr. Farber, closing.) We have occasionally seen small strands of fibrin superimposed on the membrane, but in those cases there was also a superimposed exudate.

CATAPHORETIC VELOCITY OF STREPTOCOCCI AND PNEUMOCOCCI AS ISOLATED IN STUDIES OF ACUTE COLDS, INFLUENZA AND PNEUMONIA. Edward C. Rosenow, Rochester, Minn.

Abstract. The cataphoretic velocities of the streptococci and pneumococci isolated from nasopharynx, tonsils or sputum were determined by direct observation under the microscope, in an electrical field of constant voltage, by noting the time required to traverse the unit distance of 50 microns, and are expressed in terms of microns per second, per volt per centimeter.

The cataphoretic velocities of the streptococci or pneumococci, as isolated from the different groups, were determined over an extended period, before, during, and after an epidemic of influenza, and were found to vary roughly according to the different forms of infection of the respiratory tract. The streptococci or pneumococci isolated from the nasopharynx in cases of acute rhinitis had a velocity chiefly of about 2.56 (rhinotropic); those from cases of acute pharyngitis chiefly of about 1.83 (pharyngotropic); those from cases of influenza and influenzal pneumonia chiefly of about 1.83, 1.60, 1.42 and 1.28 (influenzal and bronchotropic); and those from lobar pneumonia or croupous pneumonia chiefly of about 3.91 (pneumotropic). During the epidemic of influenza a large

proportion of normal persons and patients suffering from chronic systemic diseases became carriers of streptococci having influenzal or bronchotropic velocity, whether exposed to influenza or not.

THE PRODUCTION OF THE "G" TYPE COLONIES OF C. DIPHTHERIAE, PARK NO. 8 STRAIN. Harry E. Morton (by invitation), Philadelphia, Pa.

Abstract. Organisms resembling C. diphtheriae in morphology were obtained from Berkefeld or Chamberlain filtrates of the Park 8 strain by (1) cultivation of diphtheria toxin (confirming Hauduroy's work); (2) allowing broth cultures to evaporate to dryness, redissolving in sterile distilled water and filtering (confirming the work of Smith and Jordan); (3) forced dissociation by the presence of LiCl in the culture media (the method employed by Hadley and Richardson); and (4) serial passage in acid broth.

The "G" colonies, which are the first visible colonies obtained during the cultivation of the filterable form, may be visible only by means of the low power objective of the microscope or a hand lens magnifying fourteen times. They are at first made up of extremely short rods, some Gram-positive, some Gram-negative, and some Gram-negative containing Gram-positive granules. Upon continual cultivation on suitable media more and more of the rods became Gram-positive, increased in size to that of the normal diphtheria bacillus, and assumed the various characteristic involution forms and groupings. Fermentation reactions of the "G" forms varied greatly. The only suggestion of virulence was the development of paralysis in guinea pigs in about one month after inoculation with "G" type culture.

Discussion

(Dr. Joseph D. Aronson, Philadelphia.) I should like to ask whether or not any neutralization experiments with diphtheria antitoxin were carried out with this organism.

(Dr. Morton, closing.) No antitoxin was added to any of these emulsions.

OBSERVATIONS CONCERNING POSTMORTEM BACTERIOLOGY. Casper G. Burn (by invitation), New Haven, Conn.

Abstract. An investigation has been in progress during the past two years concerning the postmortem flora of the human body. Up to the present time there are no data available which permit an evaluation of postmortem bacteriological findings, either from the point of view of general biological interest, or of possible correlation of ante mortem bacteriology.

This report is a study based upon observations made from 300 unselected necropsies. Both aerobic and anaerobic methods were employed for cultivation of bacteria from the organs and body fluids.

The results indicate that the organs at necropsy contain a high frequency of bacteria. The strains of bacteria isolated consisted of a variety of potentially pathogenic organisms. *B. coli*, staphylococci and streptococci were the predominating groups of bacteria isolated from the organs. Since these organisms are common inhabitants of either the respiratory or intestinal tract, they may be considered as postmortem invaders. Experimental studies pertaining to this phase of the problem indicate that *B. Welchii*, *B. coli* and staphylococci were the only bacteria that successfully invaded throughout the body of the guinea

pig or rabbit after death. The dead animals were kept at room temperature (25 to 30° C) for periods of from 5 to 48 hours before autopsying and culturing. The temperature of the morgue (40° F) in which the human bodies are kept after death prevented invasion of these organisms throughout the animal body even after 72 to 96 hours. An analysis of our observations made upon human necropsies indicates that there is no significant difference in the bacteriological findings of necropsies cultured within 9 to 48 hours after death from those cultured within the first 4 hours. Therefore, postmortem invasion is not a factor in accounting for the presence of bacteria within the organs and body fluids in this group of necropsies.

Our observations reveal that certain organs contain a greater frequency of bacteria than do other organs. It is observed that cultures from the liver and kidneys contain a significantly greater number of bacteria than cultures obtained from heart blood. Heart blood cultures alone are not a true indication of the bacteriological flora of the body at necropsy. Postmortem cultures of the lungs are of little value unless there is a structural alteration that is associated with a known type of bacteria.

Further analysis of the data is in progress and will be reported in full at a later time.

Discussion

(Dr. Ward J. MacNeal, New York City.) Your icebox temperature was 40° Fahrenheit?

(Dr. Burn.) Yes.

(Dr. M. A. Kugel, New York City.) About three years ago Dr. E. Z. Epstein and I became interested in the significance of postmortem bacteriology. This study was carried out under the direction of Dr. Gregory Shwartzman. Our series was not as large as that of Dr. Burn. We examined at postmortem the blood from the inferior vena cava, the bone marrow, the heart muscle and heart valves in sixty-six cases, and were surprised at the high frequency with which organisms were recovered from these sources. This was contrary to previous findings by other workers, but the work of Dr. Burn bears out most of our original observations.

Our solid media cultures showed little or no growth. However, the material in enriched fluid media aerobically and anaerobically usually gave good results. On the basis of our studies we came to the following conclusions:

1. That no significance can be attached to the recovery at necropsy of such organisms as streptococcus alpha, streptococcus gamma, enterococcus, staphylococcus aureus, coli and pyocyaneus bacilli, unless the same organism has been recovered during life.

2. When we found unusual organisms at postmortem examination, they were of significance.

3. If we had an individual who, during life, had a specific infection, such as pneumonia, and so on, we usually recovered the infecting agent in pure culture at postmortem.

(Dr. N. W. Popoff, Rochester, N. Y.) How long do you keep your cultures before you consider them negative? From the experiments by Truffi on the development of pathologic organisms in dead tissues it is evident that both artificial and natural immunization against particular microörganisms prevents or retards the growth of such organisms in tissues, and that it is only after destruc-

tion of antagonistic substances that the microörganisms begin to grow without restriction. Have you noticed in your studies any difference as to the time of appearance and character of the growth in cadaverous tissues from individuals with a history of naturally or artificially acquired immunity toward particular organisms?

(Dr. Edwin F. Hirsch, Chicago.) In the routine examination of brains it is common to find multiple cystic cavities, and we look upon these from the practical standpoint as due to postmortem invasion of the brain by gas-producing bacteria, and do not attach particular significance to them. Is this Dr. Burn's experience?

(Dr. Joseph Aronson, Philadelphia.) I should like to ask Dr. Burn whether there is a relation between the bacterial flora and the pathological changes in the organs.

(Dr. Burn, closing.) Concerning the length of time that we retain cultures, we always keep them at least two weeks before discarding. Due to the large number of cultures made at each necropsy and the frequency of necropsies, it is essential to discard them for the purpose of obtaining incubator space.

Since our studies are incomplete in many ways, we cannot answer directly the question pertaining to antibodies in the organ at necropsy.

In regard to the relation of postmortem invasion to cysts found in the brain at necropsy, we have recently cultured twenty brains; but have found bacteria present in only four instances. The strains isolated were chiefly streptococci.

We have very frequently found bacteria in the organs without any evidence of histological changes within the organs. However, further study of our material will be necessary before anything definite can be said about this.

RETICULOCYTES AND BONE MARROW CHANGES IN PIGEONS AFTER INFECTION AND THE ADMINISTRATION OF LIVER EXTRACT. Gulli Lindh Muller (by invitation), Boston, Mass.

Abstract. To obtain some possible explanation for the failure of an adequate reticulocyte response and improvement after the administration of substances effective in pernicious anemia, to pernicious anemia patients suffering from infection, the following experiments have been carried out on pigeons, an animal peculiarly susceptible to the potent material. Groups of pigeons under standard laboratory conditions were given (*a*) staphylococcus aureus infection intramuscularly, (*b*) liver extract 343 (N. N. R.) by mouth, and (*c*) both infection and liver extract.

After infection alone there was, as a rule, during the height of the reaction, a drop in the reticulocytes from the normal level followed by an increase above normal during the period of convalescence. Active mitosis was observed in the red as well as the white blood cells of the bone marrow after 24 to 48 hours, and about the fifth to seventh day the red blood cell centers had increased enormously, indicating that infection, directly or indirectly, stimulates red blood cell formation.

Normal pigeons fed liver extract showed a characteristic reticulocyte response, but the bone marrow showed no extension of hematopoietic tissue, rather a decrease of hematopoietic activity as evidenced by a decrease of mitotic figures and a diminution in the number of megaloblasts, features observed also in patients with pernicious anemia after liver therapy.

Infection combined with the administration of liver extract resulted in a mixed reaction with the features of infection predominating.

The physiological effects on the hematopoietic organs from acute infection, therefore, seemed to be the opposite of the action of liver extract, and this may possibly explain the inhibitory effect of infection on the action of the material effective in pernicious anemia.

FURTHER STUDIES ON EXPERIMENTAL LEPROSY AND THE CULTIVATION OF B. LEPRAE. Earl B. McKinley, Washington, D. C., and Malcolm H. Soule, Ann Arbor, Mich.

Abstract. Recent work on the cultivation of *Mycobacterium leprae* and the production of experimental lesions in monkeys with both fresh leprosy tissue emulsion and cultures of the organism isolated from leprosy tissue is reviewed. Since previous reports the cultures of *M. leprae* have been carried through a total of twelve generations and are now in the thirteenth. The organism has been under artificial cultivation for over one year and has shown no tendency to grow with greater ease, but on the contrary is growing with increasing difficulty. A hormone glycerol agar has continued to be the most satisfactory medium for growth of the leprosy bacillus, and a gaseous tension of 10 per cent carbon dioxide with 40 per cent oxygen has proved most satisfactory. Various vegetable and amino-acid mediums have been employed without success. Cultures from the ninth, tenth, and eleventh generations have been tested by intradermal inoculation in older monkeys than formerly employed, and either these animals are resistant to the infection or our cultures have lost in virulence on artificial media. Serological data dealing with complement fixation and agglutination are presented, as well as a discussion concerning the mechanism of infection in leprosy in human beings and under experimental conditions.

Discussion

(Dr. Joseph D. Aronson, Philadelphia.) In association with the late Dr. Paul A. Lewis a serological study was carried out on the serum of leprous patients hospitalized at Carville, La. We found that the serum from these patients gave a positive complement fixation reaction with antigens prepared from the Duval, Kedrowsky and the Clegg strain of *M. leprae*. On the other hand, serum from tuberculous individuals, as well as from tuberculous guinea pigs and goats, reacted only with antigens prepared from the Duval and from the Kedrowsky strains, which are non-chromogenic and resemble culturally the avian type of tubercle bacilli, but failed to react with the Clegg strain, which is chromogenic. By means of absorption experiments Dr. Furth showed that the non-chromogenic Duval and Kedrowsky strains were antigenically identical with the avian type of tubercle bacillus. Since that time we have found that tuberculin prepared from these strains produces a positive tuberculin reaction when injected into the wattle of tuberculous chickens, and in tissue culture the growth of spleen and bone marrow from tuberculous chickens is inhibited; whereas tuberculin prepared from the Clegg chromogenic strain fails to produce a tuberculin reaction in tuberculous chickens and does not inhibit the growth of explants from tuberculous chickens. I should like to know how the culture isolated by Drs. McKinley and Soule differs from the Duval and Kedrowsky strains.

(Dr. McKinley, closing.) As I said, our experience on the serological side is very limited. We have not done any more work than is indicated in our report to-day. We have not compared this strain serologically with the Duval or

Kedrowsky strains. We have had many cultures of supposed *B. leprae* in the laboratory, but I cannot answer Dr. Aronson's question on the basis of any specific differences antigenically.

I should also like to mention that Dr. Wade, who was to give the next paper, and who is unable to be here, has brought from Vienna some chromogenic acidfast bacilli which Lowenstein discovered accidentally in his blood work in tuberculosis. Out of about 1000 cases he has seven which are acid-fast and chromogenic. This is the first instance I know of where a chromogenic acid-fast organism of the type described in leprosy has been isolated from non-leprous human beings, and he has seven of them. I told Dr. Wade I would mention this work to-day.

A Comparison of Typhus and Spotted Fever Rickettsiae in Tissue Cultures. Henry Pinkerton and (by invitation) G. M. Hass, Boston, Mass.

Abstract. By incubating infected tissue cultures at 32° C voluminous multiplication of both typhus and spotted fever Rickettsiae was obtained.

Typhus Rickettsiae completely fill the cytoplasm of cells which they infect. The infection spreads rapidly and by 14 to 21 days has involved practically every cell in the cultures. Large colony-like masses of organisms are found regularly in these typhus cultures. The organisms multiply only within cells, however, and the colony-like masses are found on careful study to be greatly enlarged cells distended with Rickettsiae. This condition remains constant as long as successful cultures of the cells can be maintained (up to 52 days in these experiments). Typhus Rickettsiae do not invade the nuclei of cells.

A mild strain of spotted fever (the Reimann strain) has been studied by the same technique. In this disease also the majority of cells in the cultures become infected and the organisms multiply only within cells. Morphologically and tinctorially, spotted fever Rickettsiae differ only slightly from typhus Rickettsiae. Spotted fever Rickettsiae, however, infect the cytoplasm of cells only sparsely and irregularly, but multiply massively within nuclei. Similar intranuclear massing occurs in cultures of Rumreich's eastern spotted fever. The intranuclear multiplication corresponds to that described by Wolbach in ticks infected with spotted fever, but has not previously been seen in mammalian tissues. The intranuclear clusters of spotted fever Rickettsiae are compared and contrasted with "inclusion bodies."

Comparison of the Incitants of Undulant Fever in Man and Contagious Abortion in Cattle in New York State. Ruth Gilbert and (by invitation) Marion B. Coleman, Albany, N. Y.

Abstract. Epidemiological evidence has indicated cattle or dairy products to be the source of the incitant of almost all of the undulant fever in New York, the state from which the largest number of cases was reported during 1930. Goats have not been implicated and hogs could seldom be considered. Since certain authors have attributed the majority of infections in man, in the United States, to porcine strains of the abortus-melitensis group, to which cattle are also susceptible, a study of cultures from patients in New York State is of interest. The atmospheric requirements, absorptive properties, pathogenicity for guinea pigs, behavior in the presence of dyes, and the fermentation reactions with carbohydrates of the human strains were compared with those of representative cultures obtained from other laboratories and with those isolated from milk from

cattle in various parts of the state. Almost all of the cultures from patients with undulant fever, as well as those from cattle, were found to have the characteristics generally attributed to bovine strains.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) We have carried on for the past four or five years a rather extensive study of *B. abortus* infection in our dairy herd. We have also carried on a careful study in an attempt to increase the virulence of cultures we have in the laboratory. Some of these cultures have behaved very peculiarly in that on an increase in virulence there has been a change in their agglutinability. I would like to ask the question whether it is possible at the present time to establish a clearcut distinction between the three types of *B. abortus*, the human, the porcine and the bovine. We are of the opinion that it is extremely difficult. I wonder if the change may not be due to a change in the environment, either *in vitro* in the culture media, or *in vivo* in the cattle, pigs or human beings infected. Might this not bring about a change in the type of organism, so that we cannot say this is a porcine type, and this is a bovine type?

(Miss Coleman, closing.) No attempt has been made to show that these types can be completely differentiated. Apparently it is very difficult to do so. The point which seems to be of particular interest is that practically all the strains we have isolated, whether from patients' blood or cows' milk, have behaved similarly. Also, in so far as could be determined, they have the characteristics which have been attributed to the bovine type. Whether or not the microorganisms of the abortus-melitensis group can be definitely divided into distinct types or subspecies is, I believe, questionable.

MELITENSIS MENINGO-ENCEPHALITIS. G. H. Hansmann and (by invitation) J. R. Schenken, Iowa City, Ia.

Abstract. The communication concerns a review of the localization of the Brucella group of organisms in the central nervous system. An organism of this group has been isolated from the spinal fluid of four cases. We have found no reference other than ours which deals with a complete postmortem examination. The localization may be a complication of a well marked clinical case of Malta fever, or it may be the only manifestation of the disease. Agglutinins may or may not be present in the blood. A mononuclear pleocytosis usually of less than 200 cells is an outstanding feature of the spinal fluid. Startling evanescent central nervous system symptoms such as diplopia, hemiplegia, paraplegia, convulsions, and so on, are common. Two of thirteen cases have proved fatal. The organisms may be regularly isolated from the spinal fluid if 10 cc. of fluid be used as an inoculum. The organism in our case was of the porcine variety. The pathological changes were diffuse chronic meningo-encephalitis with a diffuse arteritis at the base of the brain, which led to a mycotic aneurysm of the basilar artery. A rupture of this aneurysm resulted in death.

Discussion

(Dr. C. A. Pons, by invitation, Asbury Park, N. J.) I want to report one case of *B. abortus* meningitis observed last July in Asbury Park. There had been an outbreak of undulant fever from a dairy. The total number of cases

was nineteen. In the father of this child the only symptoms were small hemorrhages per rectum and a positive agglutination test. The family was directed to observe other members of the family. A few days later the child developed mild meningeal symptoms and spinal puncture was done. The same findings as reported by Dr. Hansmann were present; the cells numbered 250, with 95 per cent lymphocytes; the organism was isolated from the spinal fluid.

We had a positive agglutination against a stock culture of *B. abortus* in a dilution of 1:640, with the organism isolated from the spinal fluid. We obtained a positive agglutination in a dilution of 1:1800.

The child recovered after an illness of two or three weeks, but subsequently developed a myelitis, so that the child at present is paralyzed below the mid-abdomen, with loss of control of the bladder and bowels, and total paralysis of the extremities.

STRUCTURE AND BACTERIOLOGY OF SUBCUTANEOUS NODULES IN CHRONIC ARTHRITIS. B. J. Clawson, Minneapolis, Minn.

Abstract. In 300 patients with chronic arthritis subcutaneous nodules were found in 90 (30 per cent). The nodules when removed often had a definite capsule, but in some cases this was poorly defined. When sectioned and examined grossly, multiple areas of necrosis were usually seen, surrounded by fibrous tissue. Necrotic and mucinous material could frequently be expressed from the center.

The nodules were found to be made up of multiple inflammatory areas. The centers of these commonly showed varying degrees of necrosis. Two types of structure were seen in the necrotic centers, a hyaline eosin-staining material and a fibrillar substance that stained with hematoxylin. Scattered in this necrotic material were varying numbers of polymorphonuclear leucocytes. Surrounding the necrotic centers there were many mononuclear and multinucleated cells (polyblasts) which varied in size and shape. Many of them resembled the epithelioid cells in a tuberculous lesion. These polyblasts generally, but not always, had a marked tendency to be arranged in a radial or palisade fashion. In this respect the arrangement was similar to that commonly found in the heart valve in acute rheumatic endocarditis. Polymorphonuclear leucocytes were scattered among the polyblasts, in many cases in small pockets or abscesses. These nodules simulate abscesses more closely than the acute rheumatic nodules which we have studied.

Streptococci were recovered in pure culture from 70.6 per cent of the nodules cultured.

The frequency of subcutaneous nodules in acute rheumatic fever and in chronic arthritis, the similarity of the gross and microscopic structure of the nodules in these two conditions, and the frequency with which streptococci can be cultured from the blood in acute rheumatic fever and from the blood and subcutaneous nodules in chronic arthritis strongly suggest that acute rheumatic fever and chronic arthritis have a common streptococcic etiology and that the two diseases are in all probability different manifestations of the same process.

Discussion

(Dr. M. H. Dawson, by invitation, New York City.) The paper is in large part confirmation of a study which Dr. Pappenheimer and I presented before this Society two years ago. In this study we were able to show a very close

relation between the histological structure of the nodules in rheumatoid arthritis and in rheumatic fever. Last year we presented a more detailed study before the American Society of Clinical Investigation, further showing this relation. There is one point in which our studies do not correspond with those of Dr. Clawson. We were unable to obtain cultures of streptococci from the nodules. Of the organisms which we did obtain, one was a diphtheroid, and two were staphylococci. It was felt that these organisms probably represented contaminations from the skin.

"SENSITIVITY" TO SULPHYDRYL. Stanley P. Reimann, Philadelphia, Pa.

Abstract. Local areas of the skin of the arms of 450 humans were painted once with 1 per cent alcohol solution of thiocresol and controlled on the other arm with 1 per cent cresol solution. Of these, 18 individuals reacted by the production of an itching rash. The same phenomena occurred in a group of mice and rats.

Since the sulphydryl group is essential to cell division, it was thought that these individuals who are "sensitive" to this group are also "sensitive" to cell division phenomena. Adequate evidence on this point requires much more experimentation. In all probability, the reaction is allied to those by which different individuals respond to chemical groups, according to their nature and position in complex molecules.

KERNIKTERUS: JAUNDICE OF THE NUCLEAR MASSES OF THE BRAIN. H. M. Zimmerman and (by invitation) Herman Yannet, New Haven, Conn.

Abstract. Kernikterus is a condition of jaundice of various nuclear masses of the brain. The structures most commonly affected are the caudate, lenticulate, subthalamic and dentate nuclei, thalami, mammillary bodies, cornu ammonis formations, nuclei of cranial nerves, olives, parts of cerebellar cortex, and anterior and posterior horns of the spinal cord. It is most frequently, if not exclusively, associated with icterus gravis neonatorum, and its pathogenesis is through some injury to the nerve cells which are subsequently stained with bile pigments carried to them by the blood stream.

Detailed studies were made of the nervous systems of two infants who succumbed with severe neonatal jaundice, and who had kernikterus. There was, in addition, evidence of a suppurative omphalitis in each infant and of an acute hepatitis in one, from whom repeated blood cultures failed to yield any organisms. Postmortem blood cultures of the other yielded *B. coli*.

THE SUBDURAL SPACE, WITH SPECIAL REFERENCE TO SUBDURAL HEMORRHAGES. Timothy Leary, Boston, Mass.

Abstract. (*A*) *The Subdural Space:* The paradoxes which appear in connection with the subdural space reflect the differences in the origin of the pia arachnoid and the dura. The pia arachnoid, with cells derived from the neural crest (ectodermic), can limit extension of infection from the subarachnoid to the subdural space; can retain fluid in edema; can prevent invasion of meningiomata; does not organize and remove blood from the subdural space, perhaps because it is without capillaries. The dura (fibroblastic connective tissue) does not prevent extension of infection of its tissues to the subdural space; is invaded by meningiomata; organizes and removes blood from the subdural space.

These paradoxes are best explained by a concept that the subdural space is *sui generis* among so-called serous spaces; that the skull with its lining dura forms an articulation, not with bone, but with soft parts. As in other articulations a true lining endothelium is absent or discontinuous (as shown by Mallory in 1920). The pia arachnoid is an essential part of the central nervous system and is covered by a continuous layer of ectodermic (?) cells. Its relation to the dura is largely one of contiguity, with continuity only at limited points.

(*B*) *Subdural Hemorrhages:* Primary hemorrhage into the subdural space arises almost exclusively from the pia arachnoid. Only when the dura is lacerated, or when tumors arising in or invading through the dura bleed, does primary hemorrhage of dural origin occur. The sources of primary subdural hemorrhage are so closely allied to the sources of subarachnoid hemorrhage that they must be studied together. Secondary hemorrhage of dural origin, due to rupture of new vessels organizing primary collections of blood, is common. Whether the primary bleeding be due to laceration of the pia arachnoid or to the rupture of arachnoid vessels, spontaneous (from veins usually), or due to minor traumatism, the blood in the subdural space is trapped. If the arachnoid is injured, repair, shutting off the membrane with adhesion to the dura, follows promptly. The dura surrounds the clot with a thin fibroblastic membrane separating the clot from the arachnoid, and a thicker layer lining the dura. From the dural side particularly, buttresses of young tissue enclose large vascular spaces, poorly supported, which are the probable source of secondary hemorrhages within the membranous sac. The organization is from one side of the clot and therefore slow and inefficient. As in extradural hemorrhages the only adequate treatment is operative removal of the clot. The increase in head injuries associated with automobile accidents makes recognition of this condition of primary importance, particularly in patients who recover from the immediate shock, but do not progress favorably.

THE HISTOGENESIS OF ATROPHIC CIRRHOSIS. Virgil H. Moon, Philadelphia, Pa.

Abstract. Atrophic cirrhosis is essentially a progressive chronic inflammatory process. Its development may best be studied in early and active cases. Such occur in children more frequently than in adults. Sections from active cases show progressive degeneration, necrosis and dissolution of liver cells associated with an inflammatory reaction, proliferative rather than exudative in character. Whole nodules are progressively destroyed and replaced by soft connective tissue. In adjacent areas proliferation of liver cells produces expanding nodules, which compress the recently formed fibrous tissue into bands. These nodules in turn are destroyed and the process is repeated. Cirrhosis occurring in adults is usually less active. Degeneration and destruction of liver cells followed by active tissue proliferation are less marked. Occasionally complete arrest of the cirrhotic process is apparent. Such cases show no degeneration and destruction of liver cells, or proliferating fibrous tissue. Pressure atrophy from contraction of fibrous tissue may be present. Such cases may not show clinical evidence of cirrhosis. Comparison indicates that the activity of the process frequently parallels the severity of the clinical symptoms. Streptococci were cultivated from the livers of six patients with active cirrhosis. They were demonstrated in stained section in each of these, and in other cases. In none of the cirrhoses in children was there history of alcoholism. In several cases there was recent history of scarlet fever. Infection is probably one important factor in cirrhosis.

MULTIPLE MALIGNANT TUMORS. Shields Warren, Boston, Mass.

Abstract. Forty multiple malignant tumors were found among 1078 autopsies on individuals dead of malignancy. Fifteen multiple malignant tumors were found in males, who died at an average age of 67 years, and twenty-five in females, who died at an average age of 58 years. The average known duration of the older of the two tumors was 3.2 years among males and 2.8 years among females.

On the basis of Massachusetts mortality statistics, adjusted for age and sex, 5.90 cases rather than 15 would be expected in males, and 3.85 rather than 25 in females, assuming that it is admissible to accept Wilson's method of applying cancer mortality rates as morbidity rates in calculating incidence of multiple malignancy, and that the second tumor is within one year of causing death.

On the basis of a group of 12,051 malignant disease autopsies collected from the literature, 54.2 cases would be expected from a similar calculation and 111 cases were actually found.

These findings would seem to indicate that multiple malignancies occur more frequently than chance would explain, and that some constitutional susceptibility to cancer must be assumed.

Discussion

(Dr. Howard T. Karsner, Cleveland.) I should like to ask Dr. Warren whether or not he applied strictly the Billroth criteria to the multiple large intestine tumors.

(Dr. Warren.) I should have stated that in the multiple large intestine tumors I had metastases in six cases where both tumors were of intestinal origin. In the ten instances of which I spoke, they were not all ten of the intestine, but one primary tumor occurred in the intestine and the other primary tumor in some other organ. Of course we recognize that polyposis as the basis for multiple malignancy may appear frequently, but without evidence of independent metastases I did not feel justified in including those cases.

(Dr. Alfred Plaut, New York City.) I should like to ask if the older statistics have applied the Billroth criteria to tumors which often do not metastasize, like carcinoma of the cervix; that does not seem probable, because it would defeat the purpose of the statistical work from the beginning. Secondly I should like to know if Dr. Warren has received the impression from certain cases that there may be something in the constitution of a human being which leads to the formation of many kinds of tumors. Probably many of us who do autopsies cannot help having such an impression. When a middle-aged woman has a carcinoma of the cervix, a myoma uteri, multiple tumors of the peritoneum, and in addition, a primary tumor of the kidney, one cannot help but think that there must be something in this body which leads to the formation of multiple primary tumors.

(Dr. David P. Seecof, Cleveland.) I should like to ask Dr. Warren how many cases there were in which the multiple tumors produced metastases, and whether there were any in this series conforming to Billroth's criteria.

(Dr. Warren, closing.) In regard to Dr. Plaut's first question, I have not my references here, so cannot answer it exactly, but in some of the series the definite statement is made that Billroth's criteria are applied. Those series run, as one would expect, very much less than the others. In other series the matter of

Billroth's criteria is discussed, and then the statement made that they are not utilized.

With regard to susceptibility to malignant tumors, I feel quite sure that we all have the impression that there is a definite susceptibility in certain persons to cancer, and I hope on the basis of a study such as this and others similar to it that we will be able to decide that question from a statistical standpoint, as well as from the standpoint of general impression.

With regard to Dr. Seecof's question, I will say in this series there were nine cases in which there were both primary tumors and metastases of those primary tumors present. In the remainder of the cases, in practically all of them, some one of the tumors had metastasized. There were eight cases in which neither tumor had metastasized.

THE CLASSIFICATION OF TUMORS OF THE KIDNEY WITH ESPECIAL REFERENCE TO THE MALIGNANT TUMORS IN ADULTS. Baxter Lindsay Crawford, Philadelphia, Pa.

Abstract. This report is based on the study of 60 malignant tumors in adults, 4 malignant tumors in young children, and 29 benign tumors of the kidney which were discovered at autopsy. My chief interest has been in the study and classification of malignant tumors in adults. In this group of 60 cases, 59 have been classified as carcinomas and one as probably a hypernephroma. The 4 tumors in the children are either of the mixed tumor or embryonal type of carcinomas. Of the 29 benign tumors, 23 proved to be adenomas, 1 adrenal rest, and 5 fibromas or fibrolipomas. Everyone is familiar with the discussions which have appeared in the literature as to the origin of the malignant tumors of the kidney in adults since the theory was advanced by Grawitz in 1883 that these tumors arise from adrenal rests in the kidney. It is quite evident that many authors use the term "hypernephroma" to include all tumors of the kidney of a certain type, without reference to their origin. There would be much less confusion if the term "hypernephroma" were used to refer only to the ones which are considered to be of the adrenal tissue origin. It seems to be the consensus of opinion at the present time, of those who have made careful studies of large groups of these tumors, that the vast majority are true renal carcinomas and not adrenal tissue tumors. In the histological study of the majority of these tumors, true columnar epithelial cells forming indefinite acini and papillae may be demonstrated. Other points which support the view that the majority of these tumors are carcinomas instead of hypernephromas are the infrequency with which adrenal rests are found in the kidney, and the frequency with which adenomas are found in the kidney in various stages of development which may become malignant. In a series of over 2200 autopsies, I have found only one adrenal rest in the kidney, and in the same group, in something over 1 per cent of the cases, adenomas were found.

Discussion

(Dr. Ludwig Pick, by invitation, Berlin, Germany.) I am not surprised at the opposition of some people to the concept of hypernephroma, but it is a matter of fact, and not only a question of belief, that hypernephromas do exist, and I want to tell you, in addition to all other arguments, one point in support of this theory. As you all know, hypernephroma of the kidney is due to some

particles of the cortex of the adrenals which have been implanted in the kidney during fetal life. We saw one of these illustrated here — a very convincing case from the interesting material of Dr. Crawford. My argument, which has not been used until now, is as follows. You all know the so-called suprarenal tumors:

(1) The struma suprarenalis (nodules in the cortex)
(2) The tumors of the suprarenal medulla
  (*a*) Gangliocytoma and ganglioneuroma
  (*b*) Chromaffin tumors (produce adrenalin)

It has been shown that the so-called accessory suprarenals sometimes contain medullary elements. If there is really an implantation of suprarenal elements in the kidney during fetal life, such implants may also contain *a priori*, at times, cortical and medullary substance. It follows, then, that such suprarenal elements in the kidney may form either hypernephroid tumors or gangliocytoma, ganglioneuroma, or chromaffin tumors, or even combined tumors of these types. Such tumors, as I have shown, exist in the kidney, and consist of a centrum of ganglioneuroma and of a cortex of a typical so-called hypernephroma. So here we have a combination of tumors of the same organ. Therefore you see there is surely the possibility that *pure* adrenal cortical tissue may also produce hypernephroid tumors in the kidney. It is very hard to tell how often that occurs, but there is no doubt that it does occur, and all I want to emphasize is that the general meaning of the hypernephroma of Grawitz is put on a real basis.

(Dr. William Boyd, Winnipeg, Canada.) It is very dangerous to draw conclusions from one case, because it is so easy to say that it is merely a coincidence, and yet if the coincidence is sufficiently striking, perhaps it has some degree of weight. I performed an autopsy some years ago in which I happened upon such a coincidence. The patient had died of a cerebral hemorrhage. It was a straightforward case, with no suggestion of renal disease or tumor, but at the autopsy I found a most striking adrenal rest in the left kidney. I do not remember ever seeing a really striking adrenal rest in the kidney in any other case. In this respect my experience is about the same as Dr. Crawford's. I was so struck by the rest in the left kidney that I turned to those in the autopsy room and said "Wouldn't it be funny if there was a hypernephroma on the other side?" — and there was! There was a hypernephroma of the right kidney. Possibly it was a coincidence, but it appears more probable that there was some causal relation between the two.

(Dr. Crawford, closing.) The number of cases here reported is too small from which to draw definite conclusions, but the data here presented added to the many previous reports are of distinct significance. I do not mean to imply that the adrenal tumors in the kidney do not occur, for this has been definitely proved in reports of typical cases by Professor Pick and others. The points that I wish to emphasize are that in this group the majority of the specimens present definite histological characteristics of carcinoma, and that true hypernephromas are much less frequent than carcinomas of the kidney, and that the adrenal rests occur so infrequently in the kidney as to be relatively unimportant in explaining the presence of the frequent malignant tumors of the kidney in adults. It does not seem reasonable to assume that these tumors are of adrenal tissue origin when they more closely resemble the cells of the kidney, and it would seem much more probable that they arise from preëxisting adenomas.

THE DOPA REACTION IN GENERAL PATHOLOGY. George F. Laidlaw (by invitation), New York City.

Abstract. The dopa reaction is a specific stain for two kinds of cells, myelogenous leucocytes and melanoblasts. It is a valuable aid in the study of melanin production and the metastases of melanotic tumors. It identifies the actively functioning melanoblasts of the skin, of ectodermic mucous membranes, pigmented moles, melanoma and its metastases. When positive, the reaction distinguishes melanoblasts from phagocytes. A negative reaction has no meaning. The reacting substance disappears soon after death or after excision from the living body, and it is destroyed by most fixatives and preserving fluids. For melanoblasts, fresh tissue is required, or tissue that has been in 5 per cent formalin for only a few hours. Leucocytes may react after many days in 5 per cent formalin.

Discussion

(Dr. W. C. Hueper, Philadelphia.) I wonder if Dr. Laidlaw has examined the skin of the nipple. I think that the Dopa reaction should lend itself very well to settling several perplexing problems as to the origin of Paget's disease. If it is an intradermal cancer it should give a positive dopa reaction, and if it is a secondary reaction of a primary mammary gland cancer it should be negative.

(Dr. Victor Jacobsen, Albany.) This reaction should be of real service in determining the identity of cells containing brownish or brownish black pigment. We have heretofore not been able to say what a chromatoblast is and what a chromatophore is. The former cell is supposed to make pigment, and the latter only to carry it. This test apparently will settle the point. I should like to ask Dr. Laidlaw if he can throw any light by means of the dopa reaction on the actual identity of the pigmented cells which we find in the meninges, and which conceivably account for some of the malignant pigmented tumors of the meninges; also the pigmented cells of the chorioid and of the zona reticularis of the adrenal. We still have a problem ahead of us in settling the identity of intracellular pigment. Not all is gold that glitters, and probably all brownish black pigment is not melanin. We do not know what melanin is chemically. Usually it contains sulphur; it may have a little iron.

Further, how can we identify cells which do not contain pigment, but which are potentially melanin-producers? Can we take the skin of an albino animal and experimentally produce pigment by incubation for instance, which will make the cells sensitive to this test? In other words, should we consider R. Hertwig's old observation that melanin is a product of cell depression, and hence conceivably that it might be a product of practically any cell in the body? Hertwig's work was done with a unicellular organism, *Actinosphaerium eichornii*, but nevertheless I think it is of fundamental importance.

(Dr. Herbert S. Reichle, Cleveland.) I should like to ask if Dr. Laidlaw has used the dopa reaction for blood smears: I mean not only for the leucocytes in the tissues, but also for those in the circulating blood. If so, is it superior to the ordinary oxidase reaction? All of us know that the oxidase reaction often fails in the early forms of myeloblasts. Many of these do not give a positive oxidase reaction. I should like to know if the dopa reaction will give a positive reaction in such cases.

(Dr. George R. Callender, Washington.) I should like to extend Dr. Reichle's question and ask if Dr. Laidlaw has examined the pigment layer of the retina

as well as the choroid, and also the glial cells in the peripheral and central nervous systems.

(Dr. S. Weintraub, New York City, by invitation.) Have you examined myelomas?

(Dr. Joseph McFarland, Philadelphia.) I recall when Dr. Laidlaw presented the photograph of the melanoma it consisted entirely of brownish cells, and he said he could not find any signs of malignant change. I wonder whether this reaction will enable us to decide if there is a malignant change in such tumors.

(Dr. Maurice N. Richter, New York City.) Dr. Laidlaw has been kind enough to perform the dopa reaction on sections of several cases of myeloid leukemia. We have found that the dopa reaction parallels the oxidase and peroxidase reactions rather closely. Unfortunately we have not had an opportunity of examining a case of myeloid leukemia in which the cells were sufficiently young for the reaction to be of much diagnostic importance. It is my impression that myeloid cells in the early stages, the so-called myeloblasts, do not give the oxidase or peroxidase reactions until they are sufficiently differentiated to show granules by other methods when suitably stained. In one case of acute myeloid leukemia which we have had recently, in which Auer bodies appeared in the circulating blood, Dr. Laidlaw demonstrated for us an Auer body with a positive dopa reaction in the tissue sections.

(Dr. Laidlaw, closing.) Concerning Paget's disease of the nipple, I have had no opportunity to try the dopa reaction. In ordinary epitheliomas a few dopa-positive melanoblasts are often seen among the basal epithelia. I do not believe that the presence of a few melanoblasts in an epithelioma has any significance, and certainly no relation to prognosis.

I have never examined the meninges. Bloch reports the melanin-containing cells as dopa-negative in the adult, sometimes dopa-positive in the embryo.

As for the pigment of the eye, I have had no experience. It is difficult to get fresh human eyes for a dopa reaction. Such eyes are usually dropped immediately into Müller's or Zenker's fluid in the operating room. The best work on the dopa reaction in the eye was done by Miescher, of Bloch's clinic, working with chicks and rabbits. He found the dopa reaction present only during a short period of embryonic life, both in the retina and in the chorioid, while the pigment of the eye is being formed. After birth, the reaction is negative. If a malignant melanoma appears in the eye, the melanoblasts resume their embryonic activity and become dopa-positive again.

I have examined several pigmented adrenals. They were dopa-positive. The pigment was a lipoid and had nothing to do with melanin. In melanosis coli the pigment-bearing cells are dopa-negative. They are phagocytes and not melanoblasts.

I have done very little work on the blood and have formed no opinion of the value of the dopa reaction in identifying the early stages of blood cells. The subject awaits further study. In *Folia Hematologica*, of 1930, Bloch and Peck published a special technique for blood.

In regard to the nervous system, it has been reported that the ganglion cells are dopa-positive. I have been unable to confirm this. In my hands, all cells of the central and of the peripheral nervous system, both ganglion and glial cells, are dopa-negative.

The dopa reaction has no relation to malignancy, as you may see in the sec-

tions presented, where the cells of a benign mole react as strongly as the cells of a malignant melanoma. In saying that there were no signs of malignancy in the section, I meant the usual histological signs of mitosis and inflammatory reaction, not the dopa reaction.

CONCERNING THE NEURAL ORIGIN OF THE MELANOMAS. Nathan Chandler Foot, Cincinnati, Ohio.

Abstract. By means of lantern slides a series of sections are presented which very strongly confirm Masson's theories as to the neural origin of the melanomas. The photomicrographs were made from paraffin sections impregnated by the Rogers' silver method for the demonstration of neurofibrillae, as well as by methods adapted by the author, which are calculated to show the finer, connective tissue fibrillae.

Nerves and nerve terminals in normal tissue, Meissner corpuscles with their nervous apparatus, and so on, are shown; then the distribution of nerve filaments in benign melanomas is compared with these pictures and the close similarity between Masson's "lames foliacées" and the normal Meissner corpuscles is demonstrated. Besides these, more primitive fibrils and cellulofibrillar complexes are shown.

It is hoped, by means of this demonstration, that the theory of Masson is very firmly grounded and that there remains but little work to do before it may be proved conclusively. The cell nests resemble neither epidermoid nor connective tissue; they are almost invariably associated with nerve trunks; they contain numerous nerve fibers that tend to prove that the tumor cells are formed from the neural adnexa, as Masson has claimed.

ACUTE LEUKEMIA AND AGRANULOCYTOSIS. Max M. Strumia, Philadelphia, Pa.

Abstract. From the study of a large number of cases of acute leukemia, agranulocytosis and abnormal blood pictures occurring during the course of infections, especially streptococcic, the writer has previously suggested the hypothesis that there is a common causative mechanism in these various and apparently widely separated forms of blood disease.

Two cases are here presented. These must not be viewed as isolated, unusual cases, but rather as links illustrating the possible connections between acute leukemia and agranulocytosis:

Case 1 is that of a male, 21 years of age, who had, for a period of about a month during the course of an otherwise typical acute leukemia, a granulopenic phase with leukopenia as low as 400 cells per cmm.

After the granulopenic phase, the undifferentiated cells reappeared in the blood stream as rapidly as they had disappeared, the white blood count increasing in three weeks from 2100 to 430,000. The patient died with the blood showing again, as in the beginning of the disease, a typical picture of an acute leukemia.

Case 2 is that of a young girl, 9 years of age, who for six months had an agranulocytic blood picture. At this point, with only a slight increase of the total number of white blood cells (from an average of 2400 cells per cubic mm. to an average during the "leukemic" period of 3800) the patient exhibited for a period of a little over a month a typical picture of acute leukemia. This eventually disappeared and the patient had a slow recovery with a persistent moderate leukopenia and granulopenia, extending over a period of several years.

Discussion

(Dr. W. C. Heuper, Philadelphia.) Can Dr. Strumia offer any explanation for the changes in the blood picture and the condition of the patient? I should like to call his attention to the work of Lindstroem with antileucocytic serum, who was able to produce in leukemic patients such conditions as appeared spontaneously in Dr. Strumia's patient, and by the injection of antileucocytic serum in animals he could even produce the death of the animal with the histological and hematological picture of typical agranulocytosis. I have worked with antileucocytic serum in tissue cultures, and I can say that such sera have a very marked and definite cytolethal effect on leukemic and normal human leucocytes. In Wells' Textbook of Chemical Pathology is a reference to the effect of X-ray therapy on leukemia, in which it is stated that X-ray therapy increases the antileucocytic titer in the blood, and the decrease in leucocytes in the irradiated blood may be due to an increase in the antileucocytic toxin in the blood. The last case which Dr. Strumia presented seems to be more of the type of mononucleosis in which the picture is similar to leukemia, with a marked increase in the mononucleated cells. The only difference between a typical leukemia and such monocytic conditions is the prognosis. The patient recovers if he has a mononucleosis, while the patient will not recover if he has a leukemia. I once described a case where a monocytosis was present with a total leucocyte count of 88,000 for some time, and the patient recovered.

(Dr. Strumia, closing.) It is rather hard to answer this question, because Dr. Heuper did not mention whether or not he means acute leukemia or chronic leukemia. I do not think the two diseases have anything in common except the name. That might create the impression that one is a phase of the other. That is unquestionably not so.

As far as the first of the cases is concerned, we did inject rabbit serum which had been inoculated with a suspension of white cells of the patient. The injections, however, three in all, were given ten days at least after the granulopenic phase had already begun. The change is probably brought about by a variation in the toxin or toxins which are likely the cause of the condition, together with a predisposing element, preëxisting in the patient.

In regard to the question of monocytic cells, there is no doubt that in the second case we are not dealing with monocytes. The cells were carefully studied in several hundred preparations. In cases of acute leukemia monocytes rarely occur, except in the so-called monocytic acute leukemia. In this particular case there were only occasional monocytes found, and in both cases most of the cells were of myelogenous origin, in the first one showing a fairly high percentage of oxidase-positive cells, and in the second somewhat lower. These cells were not hard to recognize in the films as being of myeloid origin. For the question of effect of X-ray in leukemia, I may refer Dr. Heuper to two works which I published some time ago.*

FOCAL ARTERIOLITIS. Alfred Plaut, New York City.

Abstract. In the course of several years a peculiar, circumscribed lesion of small arteries and arterioles has been found in twenty-four patients. Two of the

* Morphologic changes of the blood in myelogenous leukemia under radium treatment. *J. Lab. & Clin. Med.*, 1924, **10**, No. 2.

On the generalized effect of radiations in myelogenous leukemia. *Am. J. M. Sc.*, 1929, **177**, 676.

lesions were found in the fallopian tube, the others in the vermiform appendix. The lesion consists of a subendothelial, hyaline deposit with necrosis of muscle coat and endothelium. The adventitia forms a granuloma consisting of spindle cells, mononuclears, irregularly round elements, and sometimes eosinophil and neutrophil granulocytes. The granuloma is well separated from the surrounding tissue. The lesion is distinctly focal. Generally several foci are found in the appendix.

There is no relation to any other disease. Only three of the patients were males, all the others females. This may be partly accidental. The age of the patients varies from 17 to 44. In fifty-nine autopsies, the appendix was examined, and in two instances focal arteriolitis was found, one patient being male. Focal arteriolitis is different from periarteritis nodosa. It is different also from the vascular lesion of typhus, and entirely different from the vascular lesions of rheumatic fever. The etiology of the disease, so far, is entirely unknown.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I am much interested in Dr. Plaut's presentation. I have not observed such lesions in human cases, but have seen them frequently in experimental animals. In a series of experiments, chronic foci of infection were produced with various organisms in order to study the prolonged effects of such infections. Focal arteriolar lesions, resembling those described, were found in a fairly high percentage of animals in which streptococci had been implanted. They were not produced by the streptococcus hemolyticus, but by streptococcus viridans, sometimes in association with lesions of the endocardium; at other times these arteriolar lesions were the only ones that indicated infection. They were found most frequently in the lungs, but were also found in cardiac muscle, intestinal walls, kidneys, and elsewhere.

(Dr. David P. Seecof, Cleveland.) Recently I have seen one case of this disease affecting every tissue in the body. That one case was found in a study of several hundred cases of arteriolar disease of the kidney. I saw this case within the last month, and it was in a human.

(Dr. Plaut.) In answer to Dr. Moon, I have to say that I have seen the lesion once — I found it accidentally in the skin of a young mouse. Nothing had been done to the mouse. I did not have any other organ of the mouse at my disposal.

As far as Dr. Seecof's remark is concerned, about arteriolar lesions of the kidney, was it a focal lesion?

(Dr. Seecof.) Yes. I have seen this lesion in the kidney often, but in this particular case every organ in the body was affected.

(Dr. Plaut, closing.) I have never seen this lesion in the kidney, and I wonder if I would recognize your lesion as identical with focal arteriolitis, or whether I should group it under arteriolar lesions we see in chronic kidney disease. Our lesion is distinctly focal. You might find a vessel involved for half a millimeter, and then it would be perfectly normal for a centimeter, and then there would be another area of involvement. There is no similarity between this lesion and arteriolo-necrosis. I have examined the spleen and pancreas of patients where we had reason to assume that the arteriolar lesion would be young, as in rapidly progressing chronic glomerulonephritis in children, but we never found anything similar to focal arteriolitis.

GLOMERULAR LESIONS ASSOCIATED WITH ENDOCARDITIS. E. T. Bell, Minneapolis, Minn.

Abstract. Two forms of glomerulitis — diffuse and embolic — are found in association with endocarditis.

Diffuse glomerulitis was found with the various types of endocarditis as follows: acute rheumatic 22.2 per cent; acute primary bacterial 28.6 per cent; subacute bacterial 64.8 per cent; secondary acute 33.3 per cent. It is characterized by an increase in the number and size of the endothelial cells and often by thickening of the capillary basement membrane. The extent of capillary obstruction is usually much less than in clinical acute glomerulonephritis, but in seven instances of subacute endocarditis glomerulitis had reached the clinical level. Diffuse glomerulitis bears some relation to the intensity and duration of septicemia.

Embolic, or focal, glomerulitis was found in the different forms of endocarditis as follows: acute rheumatic 2.9 per cent; acute primary bacterial 7.1 per cent; subacute 52.8 per cent; secondary acute 5.8 per cent. In one instance there was no endocarditis.

Two distinct types of embolic lesions occur — the fresh hyaline and the fibrous.

The fresh hyaline lesion in its simplest form is a capillary thrombosis, and all the smaller lesions are readily recognized as such. The larger lesions are composed of many thrombosed capillaries which may be identified until the capillary walls have undergone necrosis. The hyaline lesion is not an infarct, but a thrombosis and necrosis of capillaries resulting from the lodgement of bacteria. The necrotic portion of the glomerulus disintegrates and disappears.

The fibrous lesion is a reaction characterized by a marked growth of the basement membranes of the capillaries. The thickened membranes obliterate the capillaries and give the glomerulus a fibrous structure. The fibers are formed entirely from basement membranes; there is no invasion by fibroblasts from without. The fibrous lesion, like the fresh hyaline, may involve one or more lobules, or the entire glomerulus. It develops independently of the fresh hyaline lesions.

In subacute bacterial endocarditis there were fifteen instances of severe renal insufficiency, of which five were due to embolic glomerulonephritis, seven to acute, and three to chronic glomerulonephritis.

The fresh hyaline embolic lesions develop earlier than the fibrous and may be found at any time during the course of the disease. The frequent absence of embolic lesions in typical clinical examples of subacute bacterial endocarditis has not been explained.

Diffuse glomerulitis is frequently found in association with embolic lesions.

Epithelial crescents frequently cause atrophy of the glomerular tufts by compression. Fibers form between the epithelial cells and convert the crescent into a dense fibrous structure. These fibers are of epithelial origin.

In the glomeruli, fibers which later give the staining reactions of collagen are formed from three distinct sources — intracapillary fibers from the endothelial cells, fibers formed from thickened capillary basement membranes, and fibers formed by the epithelial cells of the crescents.

ACUTE DIFFUSE GLOMERULONEPHRITIS IN THE RABBIT. Kurt Semsroth (by invitation), Pittsburgh, Pa.

Abstract. Four to twenty-four hours after injection of a highly virulent pneumococcus Type 1 an acute reaction of all glomeruli of both kidneys was observed. Dominant features of the reaction were absence of early degenerative changes, enlargement of all glomeruli with capillary dilatation in the first stage, endothelial swelling and proliferation in the later stage. Since identical features set off the acute diffuse glomerulonephritis from any other kind of nephritis, the findings were interpreted as the analogue of the acute diffuse glomerulonephritis of man. Capillary dilatation was associated not with a hyperemia, but with a relative anemia of the glomeruli, while no "closing mechanism" at the vascular pole of the glomeruli was apparent. It was inferred that the primary phenomenon had been a widening of the glomerular capillary bed without a corresponding increase in blood-supply through the glomerular arteries. The observations led to the conclusion that the acute diffuse glomerulitis observed may be understood as due to the action upon the glomerular capillaries of metabolic products, which like urethane (Krogh) or histamine (Feldberg) have a dilator effect on capillaries, but none, or a constrictor effect, on arteries and arterioles.

UREA CLEARANCE FOLLOWING UNILATERAL NEPHRECTOMY. H. T. Karsner, R. A. Moore and (by invitation) R. F. Hanzal, Cleveland, O.

Abstract. The curve of urea concentration in the blood following intravenous administration of urea to unilaterally nephrectomized rabbits showed that after full recovery from operation the remaining kidney was capable of eliminating urea at the same rate as both kidneys.* In this series of experiments urea clearance of dogs was studied before, 1 month and 4 months after unilateral nephrectomy, by the method of Summerville, Hanzal and Goldblatt.† One month after unilateral nephrectomy, urea clearance under natural conditions was found to be unchanged, but if an excess of urea were administered, the urea clearance was slightly depressed as compared with the controls. At 4 months after unilateral nephrectomy, or later, urea clearance under natural conditions was not significantly changed, but with an excess of urea in the blood it was definitely increased. After full recovery from operation, and presumable increase in size of the remaining kidney, that organ was found to be capable of eliminating urea at a rate in excess of that of both kidneys before operation, a manifestation of genuine hypertrophy.

THE CELLULAR REACTIONS OF TUBERCULOSIS AND THEIR RELATION TO IMMUNITY AND SENSITIZATION. Eugene L. Opie, Philadelphia, Pa.

Abstract. The relation of tuberculosis to other forms of bacterial infection will be discussed. There is evidently wide difference of opinion concerning the significance of terms such as inflammation, exudation, and so on, as applied to tuberculosis, and even more uncertainty concerning the significance of sensitization, immunity, "allergy," and so on. With the aid of observations made

* Karsner, H. T., Straus, R., Moore, R. A., and Hanzal, R. F. Urea tolerance after unilateral nephrectomy in rabbits. *J. Exper. Med.*, 1932, **55**, 27.

† Summerville, W. W., Hanzal, R. F., and Goldblatt, H. Urea clearance in normal dogs. (In Press.) *Am. J. Physiol.*

in recent years the attempt will be made to define the conditions under which these terms can be given greater precision. The cellular reactions of first infection and reinfection will be compared and their effect upon the invading microörganisms will be discussed. The uncertainty of available means for measurement of hypersusceptibility and resistance will be pointed out. The limitations of our knowledge concerning the relation of sensitization, allergy resistance and immunity to the clinical course and pathology of human tuberculosis will be cited.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) I should like to ask Dr. Opie how he distinguishes between an ulcer formed in the skin, an ulcer formed in the intestine, and a cavity formed in the lung in a tuberculous animal. I have yet to see any evidence of ulceration in which the principal cell participating in bringing about the reaming-out of tissue is not the polymorphonuclear leucocyte.

(Dr. Opie, closing.) It is not improbable that the polynuclear leucocyte has a part in cleaning off an ulcer, particularly when, as in the intestinal tract, mixed bacterial infection occurs. It seems to me unfortunate to use the word "abscess" to designate a tuberculous cavity. An abscess is characterized by accumulation of polynuclear leucocytes which set free a sufficient amount of proteolytic enzyme to bring about solution of dead tissue and injured cells, but with a tuberculous cavity there is accumulation of epithelioid cells, or necrosis caseation and disintegration of the caseous material. Polynuclear leucocytes may penetrate into this caseous material, and have some part in bringing about its softening, but the pathogenesis of an abscess and of a tuberculous lesion proceeding to softening are so essentially different that the term abscess should not be applied to a tuberculous cavity.

CHEMICAL FACTORS IN THE EXUDATION AND NECROSIS OF TUBERCULOSIS.
Esmond R. Long, Chicago, Ill.

Abstract. It is well known that by the use of more or less purified products from the tubercle bacillus lesions can be produced in experimental animals which are closely analogous to those occurring in actual infection. These can be produced with such constancy that it is reasonable to suppose that these same substances are primarily responsible for the lesions actually observed in the disease. In recent years extensive research has been devoted to the purification of products from the tubercle bacilli and investigation of their effects in experimental animals.

The pathological effects to which injury has been directed in this study are exudation, in its widest sense, necrosis, proliferation and constitutional changes, as expressed by serum antibody reactions and related phenomena. In these effects the major fractions of the tubercle bacillus are concerned, either singly or, probably more commonly, in conjunction. In a general way the protein of the bacillus is responsible for the acute exudative phenomena of the disease, as manifested typically in the allergic reaction; the lipoids are largely concerned in the more chronic exudative manifestations and proliferative cell responses; the carbohydrates play a part in the serological antigen-antibody reactions.

The present paper deals with only a limited phase of this general problem — the more acute manifestations of exudation and necrosis. It is generally agreed

that marked exudation in tuberculosis is a reaction of reinfection, although it is admitted that enormous infecting doses, larger than those operating in actual infection, can be a primary cause of exudation. It is now well known that, in addition to actual infection, inoculation with dead bacilli will achieve a sensitiveness similar to that of infection, and it has recently been shown (Seibert) that the purified protein also will sensitize so that exudative reactions will follow its reintroduction. There is rather general agreement that a protein or protein derivative of the bacillus is the substance responsible for the exudation itself.

There is no general agreement on the etiology of the necrosis of tuberculosis, probably because of the multiplicity of factors concerned. A distinction must be drawn between acute necrosis and the more slowly developing process of caseation, but the two are frequently associated, and the first may pass into the second. According to the old view (Virchow) arising with the development of the cellular pathology, the stages in the necrosis of tuberculosis were epithelioid cell proliferation, ischemia, necrosis. Weigert considered it a form of coagulation necrosis. To-day stress is laid by many (Krause, Rich, Huebschmann, Schleussing) on the rôle of hypersensitiveness in the development of necrosis. Others (Sabin, Medlar) have stressed the relation of necrosis to the life history of certain cells. Sabin and her coworkers in particular have focussed attention on the stimulation of the monocyte by the phosphatide of the bacillus, the maturation of this cell into an epithelioid cell and its final disintegration with continued phosphatide stimulation. It is important to note that in the necrotic tissue of a tuberculous lesion several elements may be present, including the original tissue, the inflammatory exudate or proliferate, and a fibrillar ground substance, which is commonly overlooked because not brought out by the usual stains.

Acute exudation and necrosis can be readily produced in tuberculous or otherwise sensitized animals by small amounts of the purified protein obtained from the tubercle bacillus or from the culture medium on which it has grown. Quantities of this protein as small as 0.1 mg. can kill a sensitive guinea pig in eighteen hours on intraperitoneal injection. In such animals 5 to 15 cc. of exudate is poured out in the peritoneal cavity. This fluid ranges from clear to cellular and bloody, according to the intensity of the reaction. Even the clearest samples have a tendency to gel, although fibrin cannot be demonstrated in the early reactions. The fluid is alkaline (pH 7.6 or more alkaline) and rich in protein. Non-protein nitrogen is relatively high. When fixed in Zenker's fluid it fails to take fibrin stains, but stains feebly with the Mallory stain and with silver.

Intense allergic reactions can be produced with the purified protein in a variety of tissues besides the serous membranes, including the skin, testis, lung, kidney and cornea. The reaction in the latter is especially instructive. Small amounts of the protein in the cornea of a tuberculous guinea pig cause an intense exudation of polymorphonuclear leucocytes and at the same time necrosis of the connective tissue, preceded by marked swelling and loss of staining capacity of the collagenic fibers. At the same time argyrophil fibers make their appearance. These seem in large part to be due to a simple mechanical separation of the collagenic fibers by the exudate, a result in agreement with the views of Mallory and Parker on the formation of reticulin, but may be in part a new formation secondary to the degeneration and solution of the collagen in the inflammatory exudate. The presence of faintly argyrophil fibers in the anterior chamber of the eye after an intense corneal reaction with destruction of the collagenic

fibers, and an argyrophil tendency in the glue-like exudate of the peritoneal cavity, suggest that products of degenerated collagen may play an important part in the composition of exudates in tuberculosis.

Discussion

(Dr. Camille Kereszturi, New York City.) I should like to ask Dr. Long to interpret an observation of ours. We watched nine children who, after parenteral BCG vaccination, had a negative tuberculin reaction to 10 mg. OT. A few days after this test the interne made a mistake in the dilution of the tuberculin and gave 100 mg. instead of 10. To this dose all children developed a positive reaction, *i. e.*, 8 to 10 mm. erythema and infiltration with a small blister. I want to ask Dr. Long how he interprets this. Were these children slightly sensitive to tuberculin giving a negative reaction to 10 mg. of OT, or did the highly concentrated tuberculin give a mechanical reaction, a sort of foreign body reaction, or did only some component of the tuberculin produce the reaction?

(Dr. Long.) May I ask what kind of tuberculin you used for the test? That might make a difference.

(Dr. Kereszturi.) New York Health Department OT.

(Dr. Long, closing.) I have always felt that in tuberculosis there are varying degrees of sensitization that may be brought to light by varying the amount of tuberculin which is injected to produce a reaction. It would be my explanation that small amounts of tuberculin would not bring out the reaction, when the sensitization is very low, while larger amounts might induce a positive test.

I should like to say at this point that it has seemed to me that the more highly purified materials we use for the tuberculin test, the more sure we are of the results. In old tuberculin there are a great many substances of more or less unknown nature, including proteins, beef extract and peptone, as well as a large amount of glycerol and inorganic salts, and while the reactions with this material may be excellent, and the material may often behave as well in the testing treatment as other forms of tuberculin, it seems to me that when we have materials which do produce at least equally good reactions which are single pure substances, we are much more sure of the interpretation of our results than with material which contains so many unknown substances. Whether these unknown substances may be responsible for the reaction you speak of, I do not know, but I should have to consider it as a possibility.

THE SENSITIZATION OF GUINEA PIGS WITH TUBERCULIN AND THE PRODUCTION OF ANAPHYLAXIS AND ALLERGY TO THE TUBERCULO-PROTEIN. Herbert S. Reichle (by invitation) and Harry Goldblatt, Cleveland, Ohio.

Abstract. Normal guinea pigs were injected intracutaneously with 1 to 10 old tuberculin and various adjuvant substances, such as eye fluid of normal guinea pigs and horse serum. Upon retesting these animals with old tuberculin 3 to 8 days after the sensitizing injection, the animals responded in a fashion typical of bacterial allergy. Fifty-five of 102 animals used in these experiments have shown this phenomenon.

The skin reactions were of the delayed type, and although vesiculation and ulceration were never seen, they were otherwise analogous to those observed in tuberculous animals. The same reaction was obtained with Seibert's pure tuberculo-protein. At an early stage of an experiment the animals were not

sensitive to glycerin-bouillon, but after repeated injections with old tuberculin they developed sensitivity to the glycerin broth. In some of the animals the Long testicular test for allergy to tuberculin was positive; in others a strong anaphylactic sensitivity to tuberculin was demonstrated by means of the Dale test.

It is probable that an adjuvant substance is not a necessary factor and that the essential element in all previous reports of successful artificial sensitization to tuberculin has been the tuberculin itself. Some of the reasons why others have not been able to substantiate these claims are probably the failure to recognize the incubation period, the use of animals below 500 gm. and the lack at that time of an objective measure of allergy, such as the Long testicular test.

Discussion

(Dr. Max B. Lurie, Philadelphia.) Did these animals die in typical anaphylactic shock when properly tested by the antigen?

(Dr. Joseph D. Aronson, Philadelphia.) There are a number of points with which I disagree with the speaker. Old tuberculin is not antigenic and does not sensitize to itself. The late Dr. Paul A. Lewis showed that sensitization due to large amounts of tuberculin was brought about not by tuberculin, but by constituents of the broth. In so far as tuberculo-protein is concerned, its antigenic properties are different from that of old tuberculin. In his classical studies on tuberculo-protein Baldwin showed that tuberculo-protein acts as does any native antigen, and that anaphylactic shock may be produced in the non-tuberculous sensitized guinea pigs. Zinsser showed that the isolated uterus of guinea pigs sensitized with tuberculo-protein contracts when brought in contact with the antigen. We have found, as I have no doubt have other investigators, that tuberculo-protein injected into normal guinea pigs sensitizes them so that upon reinjection they die from anaphylactic shock; the isolated uterus of such sensitized guinea pigs contracts when brought into contact with the antigen. Rabbits injected with tuberculo-protein develop an Arthus phenomenon and have demonstrable antibodies in the circulating blood. In conjunction with Dr. Nicholas of the Children's Hospital we have been testing children simultaneously with old tuberculin and with tuberculo-protein. We have found that in so far as the sensitivity of the two substances is concerned, both give about the same per cent of positive reactions. However, when these children were retested three months later with tuberculin and tuberculo-protein it was found that about 40 per cent gave an Arthus type of reaction, and only about 4 per cent reacted to the old tuberculin. The Arthus type of reaction was characterized by its more prompt appearance, reaching its maximum in twenty-four hours, marked edema in a number of instances, and a subsidence of the edema after forty-eight hours. Many of the children complained of pain at the site of injection, a symptom absent when first injected. In view of our findings, I feel that tuberculo-protein as at present prepared cannot replace old tuberculin because of the danger of sensitization.

(Dr. F. B. Seibert, Chicago.) In this connection I should like to mention a few experiments which we have done this year on the purification of OT. The results which we obtained, but which I cannot describe in detail to-day, make me believe that the reactions produced by Dr. Reichle are due to the protein portion of OT. We were interested in finding whether antigenic reactions could

be obtained with any of the constituents isolated from OT similar to those produced by the highly purified tuberculin protein. Therefore we purified OT by the ultrafiltration method and obtained a colloidal solution containing protein and cabohrydrate. This, after seven to eight intracutaneous injections into a normal rabbit, gave a marked Arthus reaction, with considerable induration and hyperemia and only a little necrosis. Therefore, in OT there is still a part of the protein left intact antigenically, in spite of the hour or so of heating in its manufacture.

However, when this colloidal solution was precipitated with trichloracetic acid, a protein fraction was obtained which had about 14 per cent nitrogen and many of the characteristics of protein. Nevertheless this fraction was a poor antigen, giving scarcely any reaction following seven to eight injections into the skin of normal animals. On the other hand, it did give a reaction in the skin of tuberculous animals.

We are of the opinion, then, from this work, that it is possible to modify the tuberculin protein to some extent, as is true in the case of OT, and still get an antigenic reaction. Further chemical treatment, such as precipitation with trichloracetic acid, decreases its antigenic capacity so that it will elicit a response only in a highly sensitive animal, such as a tuberculous animal. Such a product may therefore be best for diagnostic purposes. We are very much interested in studying the chemical differences in this product and in the purified tuberculin protein.

(Dr. Reichle, closing.) In reply to the first question, we did try to inject animals intravascularly with OT. There are two objections. In the first place, it has been shown that OT, possibly because of the phenol contained in it, will in large enough doses kill normal guinea pigs. The second objection is one to which Dr. Karsner has called attention, the anaphylactoid reaction, or whatever the phenomenon may be that is connected with the injection of colloids into the vascular systems of animals. We did obtain shock in the animals. One showed typical emphysema, but we do not feel that this method is as favorable as the objective demonstration by means of the Dale method.

I do not quite understand what Dr. Aronson wishes. If he thinks that individuals injected with tuberculin may later show an anaphylactic response to tuberculin, I have no doubt that he is correct. Whether tuberculin contains protein or not, I am not in a position to say, and I must admit when I started this work we were of the opinion that it did not. Apparently Dr. Seibert's work shows that there is some, enough to produce a biological reaction. Some may prefer to call OT a haptene, although I question the validity of its use in this case.

The rôle of glycerin broth and the possibility of its giving a tuberculin reaction is such an involved subject that I think we cannot discuss it in this brief time. Within the next few years we will perhaps discover that there are special lots of glycerin broth which have some chemical and immunological relation to tuberculin. There have been many reports from Europe and America. I am not at all sure that glycerin broth cannot possibly give reactions in infected animals which are like those given by OT.

The work of Dr. Seibert I hardly need to discuss. It is a very interesting point. I wish to say that we have been in correspondence with Dr. Seibert, and that she has been very kind in giving us information and material.

A Study of the Pathogenicity of the Bacillus of Calmette-Guérin (BCG). William H. Feldman, Rochester, Minn.

Abstract. Using a strain of BCG obtained from Calmette of the Pasteur Institute a deliberate attempt was made to increase its pathogenicity by subculturing the organism on a glycerinated egg medium. Transfers were made every thirty days. From each succeeding subculture four guinea pigs were injected, two intracerebrally, one subcutaneously, and one intraperitoneally. The report deals with data obtained after the organism had been subcultured on the glycerinated egg media for fifteen generations.

Of a total of fifty-eight guinea pigs inoculated, lesions histologically indistinguishable from those of genuine tuberculosis occurred in the tissues of ten and cultures of acid-fast bacilli were obtained from each. While the majority of the lesions occurred in animals that had been injected intracerebrally, one animal injected intraperitoneally and another injected subcutaneously developed lesions of a tuberculous nature and died. So far attempts to promote a succession of tuberculous lesions by the re-inoculation into guinea pigs of infective material from lesions have failed.

Conclusions: 1. The particular strain of BCG studied is not devoid of pathogenicity for guinea pigs and the assertion that the organism is innocuous cannot be accepted without reservations.

2. Subculturing the organism on glycerinated egg medium at monthly intervals for a period of fifteen generations did not markedly enhance its virulence.

3. The state of an animal's resistance or susceptibility is perhaps of prime significance in determining whether a given individual may or may not develop tuberculous lesions following an exposure to BCG.

Protection against Tuberculosis with BCG Vaccine in Guinea Pigs. Konrad E. Birkhaug, Rochester, N. Y.

Abstract. This communication deals with a 2 year investigation of the virulence of Bacillus Calmette-Guérin (BCG), the allergic response and increased resistance against virulent tuberculous infection following vaccination of guinea pigs with the living BCG culture. My strain (BCG–Park No. 10) was subcultured according to Calmette's directions on bile-glycerine potato, glycerine potato, and Sauton's synthetic medium, as well as on Dorset's glycerine egg medium, and Petroff's gentian violet egg medium. The virulence of the whole BCG culture, as well as dissociated "S" colonies of this culture, was tested on normal guinea pigs with proper controls, by inoculating from 1 to 35 mg. (dry weight) of the culture intradermally, intraperitoneally, subcutaneously and orally. Enhancement of virulence was also attempted by Dreyer's method of deep bouillon cultures. The results of these experiments have shown that BCG retains a high degree of stability, both in cultures and in the animal body, and that my strain is completely avirulent for guinea pigs. This culture is capable of producing tuberculous lesions of purely localized nature without killing the animal for a period of 2 years after inoculation. The lesions heal spontaneously by cicatrization within 8 to 10 weeks after ulceration commences, leaving only a superficial white scar with slightly enlarged adjacent lymph nodes. The BCG strain remains viable in the caseo-purulent contents of superficial abscesses as long as 98 days after inoculation and remains avirulent when subsequently inoculated into normal guinea pigs. Cutaneous tuberculin allergy develops regularly within 2 to 4 weeks following the intradermal, intraperitoneal

and subcutaneous inoculation, reaches its maximum response between 4 and 8 weeks and may persist as long as 240 days after inoculation. The most durable and intense cutaneous allergy was produced by the intradermal inoculation of BCG and the most rapid and least stable allergy was observed following the intraperitoneal inoculation of BCG. The oral administration of 10 to 35 mg. of BCG in newly born guinea pigs yielded irregular cutaneous allergy.

Guinea pigs inoculated with BCG and subsequently, during the anergic phase, inoculated subcutaneously with a minimal infecting dose of 200 living "H_{37}" virulent tubercle bacilli, showed an increased resistance to the tuberculous infection and survived twice as long as the unprotected controls. The greatest resistance obtained in the group inoculated intradermally with BCG and the least resistance was shown in the group inoculated intraperitoneally. Thus cutaneous allergy followed closely the degree of resistance of a virulent tuberculous infection. Animals vaccinated by the oral route showed the least resistance to infection.

These investigations uphold Calmette's original contention that the BCG culture is incapable of producing progressive tuberculosis in the animal body and that it may be used without risk as a vaccine. In my series of experiments the average survival time of BCG protected guinea pigs was 50 weeks and that of the control was 27 weeks, a difference of statistical significance.

Discussion

(Dr. William H. Park, New York City.) I have been very much interested in this paper because I have been planning for some time to do what Dr. Birkhaug has done, but have not had the facilities where the animals could live in a proper way. My interest was to see whether the BCG children lose their tuberculin test, whether they are equally or less resistant, and how long that resistance lasts. I do not know whether Dr. Birkhaug can tell us how long that resistance lasts when a tuberculin injection has become negative. I think we have also come to the same conclusion that he has, that the oral vaccination was not nearly as effective as the intradermal or subcutaneous methods, and that the intradermal was possibly the best.

(Dr. Joseph Aronson, Philadelphia.) I wish to state that our results agree with those of Dr. Birkhaug. We have found that guinea pigs vaccinated either subcutaneously or intracutaneously with the BCG vaccine and subsequently infected with a virulent culture of the tubercle bacillus survive for a longer time than do control unvaccinated guinea pigs. Guinea pigs vaccinated subcutaneously with the BCG vaccine and subsequently infected intratracheally with a virulent culture of the tubercle bacillus show definite fibrosis of the hilum lymph nodes, an occasional tubercle in the lung and little or no involvement of the spleen, whereas unvaccinated guinea pigs or guinea pigs vaccinated with heat-killed cultures of the tubercle bacillus show caseous hilum lymph nodes with extensive involvement of the spleen. We have found that the R 1 strain gives results as good as the BCG vaccine.

(Dr. S. A. Petroff, Saranac Lake.) The paper presented by Dr. Birkhaug is very interesting. It supports the observations made by the majority of investigators. My interest in this organism dates back to 1925, when I began my experiment. Contrary to Calmette's claim, I observed progressive tuberculosis in guinea pigs. I was one of the first to call attention to the fact that the organism (BCG) at times, when cultivated in a certain environment, may become viru-

lent for guinea pigs. I attempted to connect the instability of this organism with the dissociation phenomenon. From the original culture I dissociated two extreme types of organisms, which differed in topography of the colonies, virulence and other biological reactions. My investigations were not corroborated until recently. We must remember that the tubercle bacillus grows very slowly and takes a long time to be modified. In 1928 I brought with me from the Pasteur Institute a culture of BCG (359). A suspension prepared as described by me was inoculated in ninety plates, and in only one of the plates three typical "S" colonies developed corresponding very closely to one which I had previously reported. From this it is evident that a great deal of patience is necessary in order to succeed in dissociating it.

Seiffert of Freiburg recently stated that at least two years are necessary to dissociate the BCG cultures. It is not surprising, then, that many in the past have failed to corroborate my claims.

Concerning immunity in guinea pigs, established after the vaccination with BCG, we failed to observe such immunity as Dr. Birkhaug reports. There is a degree of protection in vaccinated animals, but it is no greater than that established when heat-killed organisms are used. I know of no experiments, other than this one reported by him, where the protection was so great. Even the most ardent supporter of vaccination has failed to establish such immunity in guinea pigs. Professor Calmette has stated repeatedly that small laboratory animals are not suitable for testing the immunity established by this organism.

I hope that the results reported here this afternoon can be repeated and confirmed by other workers. I still believe that this method of vaccination should be a problem of the laboratory where all the evidence can be properly weighed. If the vaccine is found to be innocuous and efficient, only then should it be released for use on human beings.

(Dr. E. M. Medlar, Mt. McGregor.) In regard to BCG becoming virulent, I wish to cite one instance: about two and a half years ago, we inoculated a series of guinea pigs with BCG mixed with SiO_2. About six months ago we killed the remaining animals. None of them died of tuberculosis. There was one which had a slight enlargement of a lymph node on the same side in which the BCG had been inoculated. By inoculating this into another animal, we got progressive tuberculosis. The animal died within two months.

(Dr. Camille Kereszturi, New York City.) It is interesting that Dr. Birkhaug used egg medium. There is evidence in the literature that egg medium may increase the virulence and growth of BCG. However, in Dr. Birkhaug's experience, this did not happen, and in Dr. Park's it did not either. Dr. Park feels that the egg medium probably increases the growth of BCG and not the virulence.

A thing I should like to ask Dr. Birkhaug to do is to inject his animals in the stage in which they are positive to tuberculin, and inject others in the stage when they are negative. It is important for us to know whether we should consider children immune or relatively immune after they have lost their positive tuberculin test.

The next thing I should like to ask Dr. Birkhaug is whether he would be kind enough to try different BCG doses, because we are using much smaller amounts in children. Our highest dose is 0.3 mg. by injection. If he gives to a guinea pig 20 mg. and has good results, this might mean that we should increase our dose for children. Our chief difficulty is the determination of the ideal dose for children. Animal experiments like his might help us somewhat in estimating the desirable dosage for humans.

(Dr. Max B. Lurie, Philadelphia.) I wish to call attention to some work done in Germany recently by Tiedeman in Kirchner's laboratory, which showed that one cannot necessarily draw any conclusions from the behavior of the guinea pig as to the behavior of the human being toward BCG and other strains. For example, in the strains isolated from the Lübeck disaster, it has been found that while these strains caused the most progressive and most fulminating type of tuberculosis amongst infants, this same strain when injected into guinea pigs caused a very slowly progressive disease chiefly limited to the lymphatic system. This work was confirmed in several laboratories. The conclusion seems to be well established on the basis of other facts that the tubercle bacillus in general may be much more virulent for the human being than for the guinea pig and that the BCG also may be much more virulent for the human being than for the guinea pig.

(Dr. Park.) We have now been carrying these children for five years, and have not the slightest evidence that it does them any harm, and we have evidence that the resistance given is not so great. When they developed whooping cough, two died of the human type tuberculosis. The abscesses always heal up when they develop them.

(Dr. Birkhaug, closing.) I believe there is one cardinal difference between my experiments of protection by means of the BCG vaccine and those of other workers, in that too much haste was exercised in inoculating the virulent tubercle bacilli into the vaccinated guinea pigs before any proof was available that cutaneous allergy was established. I purposely planned to defer the superimposed virulent infection for as long as five months after the BCG vaccination, until I had tangible proof that allergy was established and most of the animals had passed into the anergic state. In this connection there seems to be little doubt now that allergy is a definite expression of immunity and that in many instances cutaneous allergy in the guinea pig is not established before eight to ten weeks following the BCG vaccination. I like to stress this significant difference between my experiments and those of other workers.

During my six months' visit last year to European centers of human vaccination with BCG, I learned something about proper dosage. The most unique results were obtained by Dr. Wallgren, at Gothenburgh, Sweden. His chief concern was to correlate dosage with production of cutaneous allergy and production of cold abscesses. His dosages were graduated from 0.2 to 0.5 mg. The latter dosage injected subcutaneously produced the least cold abscesses and the highest allergy. About 76 per cent of infants given one dose of 0.05 mg. became tuberculin hypersensitive from 4 to 9 weeks after vaccination. Another significant feature of Dr. Wallgren's series was his insistence on strict isolation of the vaccinated infant from open and virulent tuberculous infection until cutaneous allergy was established. This ideal arrangement necessarily entails financial difficulties, both for the experimental station and the parents, coupled with the disinclination of many mothers to be parted from their infants during the necessary period of isolation.

In regard to the question about the possible enhancement of virulence of the BCG organisms enough has been said. Although no living virus is absolutely fixed in virulence or avirulence, there is but most meager evidence that BCG is capable of producing progressive tuberculosis in animals or man. An overwhelming majority of workers have adequately confirmed Calmette's thesis in this respect.

The Lübeck tragedy was unique in medical annals. Again, it should be re-

emphasized that the impartial Superior Court at Lübeck clearly vindicated the harmlessness of the BCG vaccine. I believe one should be satisfied with this decision.

NEW STUDIES ON THE FILTRABILITY OF PURE CULTURES OF THE TUBERCLE GROUP OF MICROÖRGANISMS. Ralph R. Mellon and (by invitation) L. W. Fisher, Pittsburgh, Pa.

Abstract. The experiments to be reported have been designed to overcome the last remaining obstacles to the acceptance of the view that the acid-fast group of bacteria possess a filtrable phase in their life cycle. The matter is thus stated because we have employed chiefly a timothy bacillus rather than the tubercle bacillus, although even with the latter, complete parallelism exists as far as we have gone. The organism in question grows as the tubercle bacillus, is acid-fast, and is indistinguishable morphologically from this organism.

Summed up, the links in the chain of evidence are as follows: First, demonstration that the acid-fast gonidia of a granular sputum pass the filter; second, although greatly attenuated they will cause typical tuberculosis from which the virulent organism may be recovered; third, that the timothy bacillus in pure culture forms gonidia which will germinate into the original strain or one or more intermediate diphtheroid strains; fourth, that identical diphtheroid strains dissociate spontaneously without filtration; fifth, that diphtheroids with abundant acid-fast granules dissociate from pure cultures of both bovine and avian tubercle bacilli.

Discussion

(Dr. N. W. Popoff, Rochester, N. Y.) In the experimental studies on filtrability of tuberculous microörganisms the technique used is of essential importance, and for this reason the results reported here should be interpreted with great caution. In such experiments the main thing is the type of filter used. In his monograph on ultravirus Hauduroy says that filtration through porcelain bouggie, infusoria earth, caolin, and so on, must not be used any more in scientific research. The studies on tuberculous ultravirus published recently by Sanarelli and Alessandrini are of great significance. Their excellent methods discarding entirely the use of Chamberlain and Berkefeld filters ought to be considered as a fundamental prerequisite in research studies on bacterial filtrability.

(Dr. Fisher, closing.) The filters used were the Berkefeld N's and we are not assuming that they filter the normal tuberculosis organism, but rather the gonidial granules or their subdivisions. Under the conditions of the experiments we are unable to filter the tubercle bacillus itself. We do get these non-acid-fast forms after a period of incubation. We are at the present time attempting measurement of these granules by indirect methods. Our contention then is for a filtrable phase in the life history of the tubercle bacillus, biologically distinct from its "normal" acid-fast phase.

EVIDENCES OF THE NON-SPECIFIC NATURE OF THE GIANT CELL OF TUBERCULOSIS. Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. Four kinds of so-called multinucleated giant cells which have been described in tuberculosis are discussed. The common occurrence of structures morphologically resembling giant cells, but in reality only caseous foci with marginal phagocytes is demonstrated. The occasional participation of mega-

karyocytes is admitted. The evidences for and against the distinct identities of the typical Langhans' giant cells and the foreign body giant cells is weighed and the absence of dependable proofs of their separate natures emphasized. The conclusion is reached that they are probably different manifestations of one and the same cell.

Discussion

(Dr. C. A. Doan, by invitation, Columbus, O.) I do not think we differ very much with either Dr. Medlar on Dr. Haythorn in the interpretation of giant cells. We do find polymorphonuclears within the epithelioid or so-called rosette type more rarely than in the so-called foreign body type. There is less cytological differentiation in considering fixed tissues than in the supravital methods. There seems to be no question in either type of material that both fusion and mitosis occur in the development of multinucleated cells. The opportunity to observe the presence of a rosette in cells when they are stained with neutral red sharply differentiates them from those without rosette and with nuclei that are scattered diffusely through the cell, the so-called foreign body type; such criteria in a fixed preparation would not be so apparent. With reference to epithelioid giant cells, I think supravital studies show that the nuclei are restrained to the periphery because of this rosette body in the center of the cells. In the very earliest tubercle made up of epithelioid cells, which are proliferating rapidly, it has been our experience in supravital studies that the so-called foreign body type of giant cell is exceedingly rare. In the early stages before there has been necrosis with attendant foreign body reaction, only the smaller multinucleated cells with central rosette are present. There is no question, however, that later on we have a mixture of both types of cells, or the two morphological expressions of one type, whichever interpretation you care to make.

THE EFFECT OF VIRULENCE OF TUBERCLE BACILLI ON THE HISTOPATHOLOGY OF TUBERCULOUS LESIONS IN NORMAL ANIMALS. E. M. Medlar and (by invitation) K. T. Sasano, Mt. McGregor, N. Y.

Abstract. This study was made with two strains of tubercle bacilli, one of bovine origin and one of avian origin. In each instance we had cultures of high and of low pathogenicity of the same strain. The animals were all inoculated intravenously. In the case of the cultures of high virulence all of the animals died within two months. In the animals inoculated with bacilli of low pathogenicity none died even if allowed to live as long as two years.

The essential differences in the pathology are as follows: The chief lesions produced by the bovine cultures of high virulence were abscesses which ruptured and caused a "spreading" of the disease and by the virulent avian bacilli of extensive mononuclear infiltration with necrosis of these cells and subsequent neutrophilic infiltration. The chief lesions produced by the bacilli of low pathogenicity were mononuclear tubercles, often with giant cells present; collections of giant cells, often with pigment; large collections of lymphocytes, and scars with little lymphocytic infiltration.

From these studies it is evident that the typical text-book description of "tubercle" represents a retrogressive or healing phase of tuberculosis. The same is true of lesions where scar tissue and lymphocytic infiltration predominate. Such lesions represent the pathology in normal animals infected with bacilli of low pathogenicity or the lesions of successful resistance in animals or human beings infected with bacilli of high virulence. Bacilli are absent or extremely rare in such lesions.

It is evident also that abscesses and caseation, which is essentially a dead abscess, represent the more serious and dangerous lesions in normal animals and in human beings, and animals with increased resistance infected with bacilli of high virulence. It is the formation of these abscesses with their subsequent rupture that leads to cavity formation, hemorrhage and serious "spreading" of the disease. The participation of the neutrophil in the tuberculous process as the predominant cell type indicates the most serious type of infection. Bacilli are always abundant in such lesions except in the old caseous foci.

Discussion

(Dr. Max B. Lurie, Philadelphia.) In regard to the correlation which Dr. Medlar has brought out between the polynuclear leucocyte and the tubercle bacilli, I wish to say we have had the opportunity of following a strain of tubercle bacilli which is essentially the same type as Dr. Medlar has been using, and we have followed the number of bacilli present in the lesions, not by the number of tubercle bacilli which can be stained, but by the actual number of living bacilli present, as determined by culture, and it was found that the numbers of tubercle bacilli were greater before there was any infiltration of polynuclear leucocytes, and that with this particular strain of tubercle bacilli, a strain which grows with greatest rapidity in the rabbit body, the tubercle bacilli were greatly reduced in number when the first stages of caseation and infiltration of polynuclears set in. One reason for the appearance of the polynuclears in the lesion is the death of the cells and the release of the remaining contained bacilli.

(Dr. N. W. Popoff, Rochester, N. Y.) Have you noticed any definite relation between the type of cytological, experimentally produced change, and the picture of the blood?

(Dr. Medlar, closing.) The question of trying to determine from sections whether tubercle bacilli are living or not is of course impossible. All I can say is that during the first process where the monocyte is predominant, there is an increase of tubercle bacilli which causes disintegration of the monocytes. Subsequent to the degeneration of the monocytes there occurs an influx of the polynuclear leucocyte. Whether tubercle bacilli are killed before the polymorphonuclear leucocytes get in, I do not know, but certainly we get a tremendous increase of stainable bacilli when the sections are full of polymorphonuclears.

In regard to the question about the blood, I may say that for the last five years I have been doing a large number of leucocyte counts; we have had over 30,000 of them, and we have followed a great many tuberculous cases by weekly counts for a year or more. We have done studies on rabbits also. I have not seen any case which had progressive tuberculosis in which the neutrophil was not increased in the circulation. The percentage of neutrophils may be normal, but in such instances one may find a larger number of immature neutrophils in the circulation than of the mature.

CHANGES WHICH OCCUR IN THE TUBERCLE BACILLUS IN RELATION TO THE DEVELOPING TUBERCLE. C. Eugene Woodruff, Nashville, Tenn.

Abstract. Following intraperitoneal inoculation of tubercle bacilli in the guinea pig the bacilli collect in large part in the omentum. By studying "spread" inoculations of the omentum, stained by Ziehl-Neelsen's method, morphological changes in the bacilli have been identified. After 48 hours the bacilli are found bunched in certain cells — usually in polymorphonuclears or monocytes,

but occasionally in cells of the omentum with no sign of inflammatory cells about them. Six days after inoculation the bacilli which were seen in cells of the omentum have undergone multiplication, as indicated by great masses of bacilli arranged in parallel chains. There is still no sign of inflammatory reaction about them. After 10 days the large masses of bacilli are no longer found in the omental cells, having been included, possibly, in the newly formed tubercles. At 14 days the acid-fast bacilli, which are now found only in or about tubercles, stain faintly. Also at this period there appear about the larger tubercles minute granular structures which are sometimes acid-fast and sometimes non-acid-fast. After 17 days the tubercles and the tissues surrounding them contain many long, beaded bacilli which stain a bright red.

FURTHER STUDIES ON THE SURVIVAL TIME OF TUBERCULOUS RABBITS INJECTED WITH FERRIC CHLORIDE. Valy Menkin, Boston, Mass.

Abstract. Previous experiments have shown that repeated intravenous injections of ferric chloride (0.25 per cent) are followed by an accumulation of iron in the caseous areas of tuberculous animals. Concomitantly with this accumulation it was found in a series of 16 tuberculous rabbits that there was a marked increase in the survival time as compared with that in the controls. These studies have been repeated and extended with some modification of technique in the present series, consisting of 36 rabbits, with the following results:

1. The survival time of 10 control brindle rabbits ranged from 71 to 124 days with an average of 94 days. The survival time of 10 experimental brindle rabbits ranged from 89 to 190 days with an average of 135 days. The average increase in the experimental group was therefore 41 days, as compared with 48 days in the previous series.

2. The average weight plotted against time showed a slight increase in weight in the experimental animals during part of the period of ferric chloride injections. This was followed by a gradual fall in weight, terminating in the death of the last experimental rabbit the 28th week after inoculation with tubercle bacilli. The control group showed no average increase in weight during the period when the experimental animals were receiving ferric chloride injections. At the 10th week a sharp fall in weight began to take place, terminating in the death of the last of the control rabbits by the 17th week.

3. A comparison of the pathological appearance of the lungs revealed fewer tuberculous foci in the 4 shortest experimental survivors (89 to 112 days) than in the 4 shortest survivors of the control group (71 to 79 days).

4. In a parallel series of 24 tuberculous rabbits the latter were either killed at various intervals, or, in a few cases, allowed to die of their disease. This was done in order to compare the progress of the disease, as indicated by the extent of the pulmonary lesions, in the control and in the experimental rabbits injected repeatedly with ferric chloride, when both groups of animals had had the disease for about the same length of time. The interval between the injection of tubercle bacilli and the death of the animals ranged from 45 to 97 days. In 83 per cent of the animals, the tuberculous lesions in the lungs were distinctly less developed in the experimental group than in the control group. These results indicate that repeated ferric chloride injections retard the progress of tuberculous lesions in the lungs of rabbits infected with a bovine strain of tubercle bacilli.

5. In a short preliminary series of 15 tuberculous rabbits, 8 of which had received a total of 45 cc. of 0.25 per cent ferric chloride followed by a total of

22 cc. of 5 per cent $CaCl_2$, only a relatively slight increase in the survival time was found in the experimental group of tuberculous rabbits amounting to about 27 per cent over that of the controls. Further studies are now in progress on this phase of the problem.

6. *In vitro* studies show that when to a saline suspension of Ravenal bovine tubercle bacilli 0.25 per cent of ferric chloride is added, a flocculent yellowish precipitate (probably a ferric proteinate) sinks to the bottom of the test tube, leaving a clear supernatant fluid.

The data in the published and in the present studies of tuberculous rabbits repeatedly injected intravenously for 10 and 20 weeks respectively with 0.25 per cent ferric chloride show that concomitantly with an accumulation of iron in caseous areas there is a retardation in the development of tuberculous lesions in the lungs, as well as a significant increase in the survival time of experimental animals.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) I should like to ask whether careful histological studies were made of any of the lesions, because if this treatment has a beneficial effect on the tuberculous lesions, certainly the lesions from animals so treated should be very different histologically from those in the untreated animals.

(Dr. C. A. Doan, Columbus.) Were any cytological studies of the blood, particularly of the red cells, made? There is a definite decrease in the color index in chronic tuberculosis, and I wonder if these injections of iron had any influence upon this phenomenon in the peripheral circulation itself.

(Dr. Menkin, closing.) In answer to Dr. Medlar's question, we have not yet studied these lesions histologically in a very extensive manner, but in some cases that we have studied, however, the type of tubercle is very much like those shown here to-day, with the usual caseation in the center, and mononuclear phagocytic response at the periphery. We were interested to know whether there would be a greater fibroblastic proliferation about the lesions, but I was unable to find anything very consistent; some lesions showed at their periphery a great deal of vascular engorgement, but we also found that in some of the controls.

In reference of Dr. Doan's question, I think that the results of our experiments indicate that concomitantly with the accumulation of iron in tuberculous areas there is a prolongation in the survival time of experimental animals, but it is conceivable at the same time that there may be other factors concerned in explaining the effects on the course of the disease besides the accumulation of iron in tuberculous foci. I have done hemoglobin determinations and total leucocyte counts for several weeks on two rabbits, and I have found no appreciable change, but I have as yet made no studies of the color index.

THE PERSISTENCE OF TUBERCULOUS INFECTIONS. H. E. Robertson, Rochester, Minn.

Abstract. Routine studies of tuberculous foci examined by stained sections show a surprising number in which signs of latent or persistent activity of the infection are indubitably present. In many such cases there has never been even a suspicion of the disease during life. The study strengthens the assumption "once infected (with tuberculosis) always infected," which is a safe but probably not a wholly true generalization.

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